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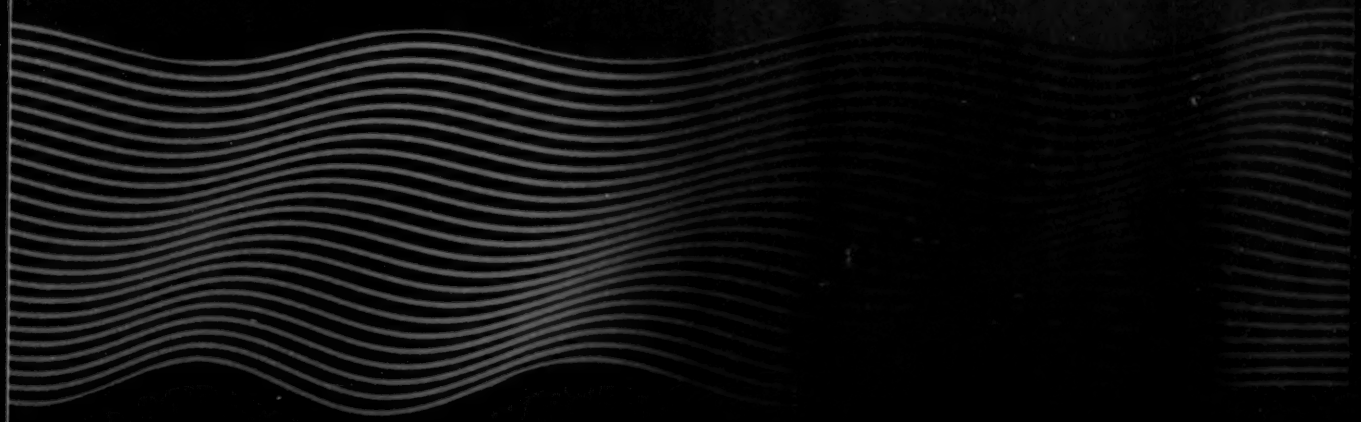
Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques



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CJFAS has had a long tradition of publishing the proceedings of symposia in special issues and supplements of the Journal. We would like to inform our readers that this practice has not changed and to remind you of the basic guidelines for the publication of these collections of manuscripts.

Organizers of symposia should contact the Editors well in advance of the symposium date, detailing such information as the theme and scope of the symposium, as well as the expected number of contributions. A typical supplement contains 20–25 manuscripts, though larger groups of papers are acceptable. Proceedings based on fewer papers may be published as a group, along with other peer-reviewed manuscripts, in a regular issue of the Journal. Once the Editors are in general agreement that the collection of manuscripts meets CJFAS Editorial policy, symposia organizers will need to make financial arrangements with NRC Research Press. Depending upon the subject and deemed importance to the readers of CJFAS, there may be an opportunity to waive page charges partially or in entirety.

Symposium proceedings are peer reviewed with the same degree of rigor as regular Journal submissions. For groups of papers greater than 10–12, a common practice is to invite the symposia organizers to act as Guest Associate Editors during the review process. The Journal office typically handles the initial stages of the review process involving the acquisition of reviews. Although Guest Associate Editors are required to suggest the names of 2–3 potential referees per manuscript, the final selection of referees remains with the Editors. Guest Associate Editors will be asked to evaluate the reviews of each manuscript and make any additional comments, as they deem necessary. This evaluation of reviews includes the arbitration of divergent reviews, should the referees differ in their opinion of a particular manuscript. After peer review has been completed, the Guest Associate Editors are asked to correspond directly with the authors, and they are responsible for the coordination of revisions. Only when the manuscript is in final, publishable form do they return the works to the Editorial office.

For further details on CJFAS supplements and special issues, and for a complete set of guidelines, please contact the Editorial office.

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La publication de numéros spéciaux et de suppléments

Le JCSHA a l'habitude de publier les actes des symposiums dans des numéros spéciaux et des suppléments. Nous voulons aviser nos lecteurs que cette pratique n'a pas changé et vous rappeler les lignes directrices de base concernant la publication de cette collection de manuscrits.

On demande aux organisateurs du symposium de communiquer avec les éditeurs bien avant la tenue du symposium, afin de les informer du thème et de la portée de celui-ci, ainsi que du nombre de communications prévues. Habituellement, un supplément contient de 20 à 25 manuscrits, mais il est possible d'augmenter ce nombre. On peut regrouper un plus petit nombre de manuscrits et les publier avec d'autres manuscrits examinés par les pairs dans un numéro ordinaire. Quand les éditeurs conviennent que les manuscrits correspondent à la politique éditoriale du JCSHA, les organisateurs du symposium doivent conclure un accord financier avec les Presses scientifiques du CNRC. Selon le sujet et son importance pour les lecteurs du JCSHA, on pourra supprimer partiellement ou entièrement les coûts par page.

Les actes du symposium sont examinés par les pairs aussi rigoureusement que le sont les autres textes soumis au Journal à des fins de publication. Lorsque le nombre de communications groupées dépasse 10 ou 12, on invite habituellement les organisateurs du symposium à jouer le rôle de rédacteur adjoint invité pendant l'examen. Le Journal s'occupe des premières phases du processus, liées à la réalisation des examens. On demande aux rédacteurs adjoints invités de suggérer le nom de deux ou trois lecteurs par manuscrit, mais le choix final des lecteurs revient aux éditeurs. Les rédacteurs adjoints invités devront évaluer les examens effectués pour chaque manuscrit et faire des commentaires supplémentaires s'il le juge nécessaire. L'évaluation des examens comprend l'arbitrage des divergences lorsque les lecteurs n'ont pas la même opinion au sujet d'un manuscrit. Quand l'examen par les pairs est terminé, les rédacteurs adjoint invités communiquent directement avec les auteurs et coordonnent l'apport des corrections. Ils ne remettront au bureau de la rédaction que la version finale des manuscrits, prête à être publiée.

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http://www2.ucsc.edu/people/msmangel/Krill_Symposium.html

The Second International Symposium on Krill

We are pleased to announce the Second International Symposium on Krill, **23–27 August 1999** on the campus of the University of California Santa Cruz.

The purpose of this symposium is to review current knowledge of krill euphausiid biology, to highlight gaps and future areas for research, and to show how the study of a range species of krill can illuminate more general problems (for example, krill flux, recruitment, swarming behaviour, age, and growth). The sessions (and invited speakers) are

- **Krill demography, life history, and genetic diversity** (V. Siegel, Germany; Vassily Spirodonov, Germany; T. Paternello, Italy; E. Pakamov, South Africa)
- **Krill development, growth, reproduction, and aging** (N. Iguchi, Japan; S. Nicol, Australia; J. Cuzin-Roudy, France)
- **Krill physiology, metabolism nutrition, and energetics** (L. Quetin, U.S.A.; F. Buchholz, Germany; R. Perissinoto, South Africa; Y. Nishino, Japan; P. Mayzaud, France; S. Falk-Petersen, Norway; A. Clarke, U.K.)
- **Krill behavior: swarming, vertical migration, foraging, and antipredator mechanisms** (W. Hamner, U.S.A.; D. Ritz, Australia; L. Madureira, Brazil; J Watkins, U.K.; A. Brierley, U.K.)

The opening talk will be given by D. Miller (South Africa).

Each day will include invited and contributed talks and poster sessions. Proceedings of the meeting will be published; contributors will be expected to arrive with disk and hard copies of their papers.

A call for contributed talks will occur in fall 1998. Special support will be available for young investigators and students.

Further communication will occur electronically, either at the web site **http://www2.ucsc.edu/people/msmangel/Krill_Symposium.html** or by contacting Marc Mangel by e-mail (msmangel@cats.ucsc.edu).

International Organizing Committee: Marc Mangel, Steve Nicol, Janine Cuzin-Roudy, Yoshi Endo, Denzil Miller, Robin Ross, Völker Siegel, and Jon Watkins

Local Host Committee: Sarah Barnett, Don Croll, Marc Mangel, and Baldo Marinovic

Le deuxième symposium international sur le krill

Nous sommes heureux d'annoncer la tenue du deuxième symposium international sur le krill, **du 23 au 27 août 1999,** sur le campus de l'Université de Californie, Santa Cruz.

Le symposium a pour objet d'examiner les connaissances actuelles sur la biologie du krill, de souligner les lacunes et les futurs sujets de recherche et de montrer comment l'étude de diverses espèces de krill peut éclairer des problèmes plus généraux (par exemple le flux, le recrutement, l'essaimage, l'âge et la croissance du krill). Voici les titres des exposés, accompagnés des noms des conférenciers invités.

- **Démographie, cycle biologique et diversité génétique du krill** (V. Siegel, Allemagne; Vassily Spirodonov, Allemagne; T. Paternello, Italie; E. Pakamov, Afrique du Sud)
- **Développement, croissance, reproduction et vieillissement du krill** (N. Iguchi, Japon; S. Nicol, Australie; J. Cuzin-Roudy, France)
- **Physiologie, métabolisme, nutrition et énergétique du krill** (L. Quetin, É.-U.; F. Buchholz, Allemagne; R. Perissinoto, Afrique du Sud; Y. Nishino, Japon; P. Mayzaud, France; S. Falk-Petersen, Norvège; A. Clarke, R.U.)
- **Comportement du krill: essaimage, migration verticale, quête de nourriture et protection contre les prédateurs** (W. Hamner, É.-U.; D. Ritz, Australie; L. Madureira, Brésil; J. Watkins, R.U.; A. Brierley, R.U.)

M. D. Miller (Afrique du Sud) prononcera le discours d'ouverture.

Des communications sollicitées, des communications offertes et des communications affichées seront présentées chaque jour. Les actes du symposium seront publiés; c'est pourquoi l'on demande aux conférenciers de copier leur communication sur disquette et sur copie papier.

À l'automne 1998, on lancera une invitation aux personnes intéressées à présenter des communications offertes. Un soutien spécial sera accordé aux jeunes chercheurs et aux étudiants.

À l'avenir, les renseignements seront transmis par voie électronique, soit par l'intermédiaire du site Web **http://www2.ucsc.edu/people/msmangel/Krill_Symposium.html** soit par courrier électronique (Marc Mangel : msmangel@cats.ucsc.edu).

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Partitioning the influence of physical processes on the estimation of ichthyoplankton mortality rates. I. Theory

J.A. Helbig and P. Pepin

Abstract: This paper examines the effects of natural spatial–temporal variability in ichthyoplankton concentration and in currents on the estimation of mortality rates. We derive expressions for the biases and variances of mortality estimates computed from the change in plankton concentration between successive surveys as well as estimates of the corrections due to the advection of plankton. We demonstrate that estimate bias depends primarily on how well the mean current and mean ichthyoplankton fields are sampled whereas the variance depends on the variability about the mean in currents and plankton concentration and on the time and length scales on which this variability occurs. Simplified versions of the theoretical expressions provide an easily implemented framework for evaluating the quality of field sampling plans.

Résumé : La présente communication traite des effets de la variabilité spatio-temporelle naturelle de la concentration d'ichtyoplancton et des courants sur l'estimation du taux de mortalité. Nous avons obtenu des expressions mathématiques des biais et des variances de la mortalité estimée calculée à partir de la variation de la concentration planctonique entre des relevés successifs de même que des estimations des corrections découlant de l'advection du plancton. Nous montrons que le biais de l'estimation dépend surtout de la qualité de l'échantillonnage du courant moyen et des concentrations moyennes de l'ichtyoplancton tandis que la variance est fonction de la variabilité par rapport à la moyenne des courants et de la concentration du plancton et des échelles de temps et de distance subissant l'effet de cette variabilité. Des versions simplifiées des expressions théoriques constituent une approche d'application facile pour l'évaluation de la qualité des plans d'échantillonnage sur le terrain.

[Traduit par la Rédaction]

Introduction

Despite evidence that events prior to the juvenile stage are important elements of marine fish population dynamics (e.g., Myers and Cadigan 1993), our understanding of the processes that contribute to the loss of fish eggs and plankton is limited. Research has focussed on the effects of predator–prey interactions on early life survival (Heath 1992; Leggett and Deblois 1994) and shows a change in mortality rates consistent with predictions of energy flow in pelagic ecosystems (Peterson and Wroblewski 1984; Pepin 1991). However, very little empirical evidence from individual field studies supports this conclusion (Heath 1992; Leggett and Deblois 1994). We perceive an underlying belief that food web dynamics are the primary source of variation in survival (Bailey and Houde 1989; Heath 1992) and that spawning patterns ensure life history closure. However, if life histories have evolved such that patterns of drift or retention ensure development within specific habitats or food webs, does that not also make them vulnerable to variations in those same currents that are assumed to limit dispersal?

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Few attempts have been made to directly measure the contribution of transport to changes in population abundance (Taggart and Leggett 1987; McGurk 1989; Reed et al. 1989; Kim and Bang 1990; Hinckley et al. 1993; Pepin et al. 1995). Taggart and Frank (1990) suggested that estimated mortality rates of fish eggs and plankton could be biased due to immigration, and they proposed that the bias would be scale dependent. Similarly, Pepin et al. (1995) presented a simple scaling argument suggesting that the relative importance of advective losses should increase as the area of the study site decreases. Both Taggart and Frank (1990) and Pepin et al. (1995) emphasized the concept that the time and space scales of field studies/surveys are fundamental factors that influence the measurement of ichthyoplankton vital rates because of the influence of physical processes.

It is informative to undertake an exploratory analysis aimed at comparing various mortality estimates made from field studies with theoretical predictions to see if this view has much empirical support. Mortality rates estimated from both fixed-area and patch studies exhibit a decrease in magnitude with increasing size of the study area (Fig. 1). Estimates of advective losses show nearly the same scale dependence, in rough agreement with the prediction of Pepin et al. (1995). By contrast, mortality estimates based on predatory losses alone show no such dependence. However, fitting all three data sets simultaneously (see legend to Fig. 1) indicates that mortality estimates show a scale dependency in the relative contribution of advective and predatory losses. The similarity of the dependence of total mortality

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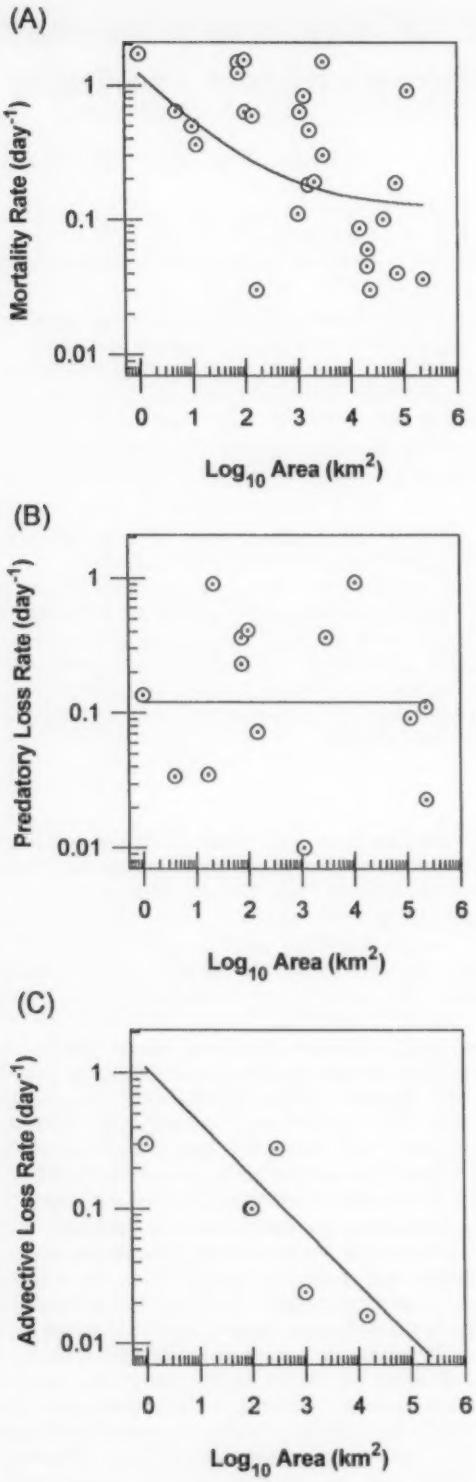

Fig. 1. Estimates of (A) total, (B) predatory, and (C) advective losses measured in various studies of fish eggs and larvae. The lines shown in each panel represent the relationships estimated based on minimizing a combined nonlinear sum of squares such that

$$SS = \sum_{\gamma}(aA + bA^c - M)^2 + \sum_{\alpha}(aA - P)^2 + \sum_{\beta}(bA^c - V)^2$$

where M, P, and V are the total, predatory, and advective losses, γ, α, and β represent the independent data sets for each of those losses, A represents the area of the study site, and a, b, and c are estimated parameters with values of 0.12, 1.10, and –0.40, respectively. Analysis revealed no evidence of a significant exponential slope in predatory losses. Some of the following sources provided estimates of losses attributed to more than one factor. Sources for total losses: Talbot 1977; Lough et al. 1981; Santander et al. 1983; Anderson 1984; Chavance et al. 1984; Henderson et al. 1984; Fortier and Leggett 1985; Hewitt et al. 1985; Ware and Lambert 1985; Heath and MacLachlan 1987; Leak and Houde 1987; Taggart and Leggett 1987; Kiørboe et al. 1988; Peebles and Tolley 1988; Kim and Bang 1990; Castro and Cowen 1991; Davis et al. 1991; Cowan and Houde 1993; Hinckley et al. 1993; Theilacker et al. 1993; Houde et al. 1994; Murdoch and Quigley 1994; Purcell et al. 1994; Pepin et al. 1995. Sources for predatory losses: Möller 1980; Arai and Hay 1982; Santander et al. 1983; Purcell 1984, 1989; Daan et al. 1985; van der Veer and Bergman 1987; deLafontaine and Leggett 1988; Fancett and Jenkins 1988; Cowan and Houde 1993; Theilacker et al. 1993; Houde et al. 1994; Purcell et al. 1994. Sources for advective losses: Fortier and Leggett 1985; Taggart and Leggett 1987; McGurk 1989; Kim and Bang 1990; Davis et al. 1991; Pepin et al. 1995.

rates with advective losses at small spatial scales suggests that many mortality estimates may be corrupted by transport effects, thus limiting inferences about other regulatory processes.

Although this analysis suggests that transport may be an important consideration, no quantitative assessment of the accuracy and precision of transport estimates has been undertaken. The objectives of this study were (*i*) to develop a framework for estimating the relative importance of transport in systems of varying size, (*ii*) to develop criteria for survey design prior to field studies that take into account expected environmental variability, and (*iii*) to determine how transport-induced bias and variance in mortality estimates affect our ability to answer biological questions about the early life history of fish plankton. A key feature of this study is that we provide a framework that takes into consideration the structure of currents and eddies, which are critical elements influencing the dispersion of plankton (Power 1996; Pepin and Helbig 1997), in contrast with other approaches based on simple advection–diffusion schemes.

Plankton balance

As the least complicated system in which physical processes influence the estimation of mortality rates, we consider a rectangular basin separated from the deep ocean by a single open boundary (Fig. 2) in which we attempt to measure changes in abundance from two surveys separated in time by ΔT. We consider experiments in which plankton are sampled over a fixed depth h deep enough such that their concentration at the bottom of the tow vanishes. Let the hor-

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izontal coordinate $\mathbf{x} = (x,y)$ be such that x points across and y points out of the basin and let $\mathbf{u} = (u,v)$ be the corresponding vertically averaged horizontal velocity. (Vectors are denoted in bold (Table 1)). Then the balance of plankton at any point (x,y) within the basin is

$$(1) \qquad \frac{\partial c}{\partial t} = -Zc - \nabla \cdot (\mathbf{u}c) + K_H \nabla^2 c$$

where $c(x,y,t)$ is the vertically averaged plankton concentration (number per cubic metre), Z is the biological mortality, and K_H is the horizontal diffusivity. The left-hand side of eq. 1 represents the total change in abundance at any point and includes losses due to biological mortality plus apparent contributions to mortality due to physical transport. Differential mortality is avoided by prescribing Z as constant in space and time. Thus, any variations in estimates of Z considered here are due to effects associated solely with the physical environment and with measurement error.

In deriving eq. 1, we have assumed that the vertical variation in $\mathbf{u}$ and c is small enough so that the vertical average of their product is adequately represented by the product of their averages. In systems in which plankton actively migrate vertically (e.g., to exploit tidal currents) the present theory would require some modification, although the findings presented here would not be greatly affected.

Equation 1 can be averaged over the basin area to obtain an expression that is readily applied to most field data:

$$(2) \qquad \frac{d\langle c(t)\rangle}{dt} = -Z\langle c(t)\rangle - \frac{1}{A}\int_{L_x} v(x, L_y, t)c(x, L_y, t)\,dx + \frac{K_H}{A}\int_{L_x} \frac{\partial c(x, L_y, t)}{\partial y}\,dx.$$

Here, the angle brackets denote an areal average:

$$(3) \qquad \langle c(t)\rangle = \frac{1}{A}\int_A c(x,y,t)\,dxdy$$

where L_x and L_y are the basin width and length, respectively, and $A = L_x L_y$ is the basin area. To avoid the clutter of multiple integral signs and limits, we denote the integral type by a subscript; for example, $\int_A (\cdot)\,dxdy$ signifies the integral over the basin area.

In order to investigate the effects of environmental variability, we model both the velocity and plankton concentration fields stochastically. In any given experiment, the velocity field is regarded as one possible realization drawn from an ensemble of fields constrained only by a very limited knowledge of the true field. Since the plankton are assumed to drift passively, the plankton field is also stochastic although not independent of the velocity field. We put

$$(4) \qquad \mathbf{u}(\mathbf{x},t) = \mathbf{u}_o(\mathbf{x}) + \mathbf{u}'(\mathbf{x},t)$$

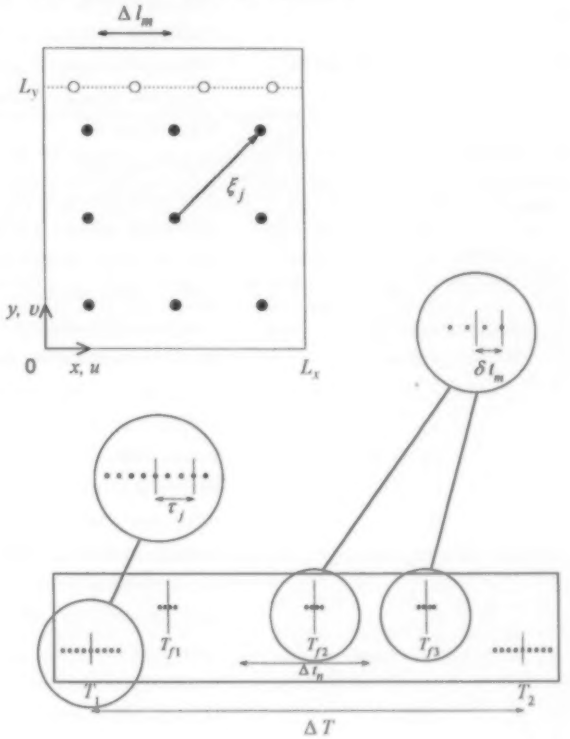
$$(5) \qquad c(\mathbf{x},t) = c_o(\mathbf{x},t) + c'(\mathbf{x},t)$$

with

$$(6) \qquad \mathcal{E}(c_o) = c_o,\ \mathcal{E}(\mathbf{u}_o) = \mathbf{u}_o,\ \mathcal{E}(c') = 0,\ \mathcal{E}(\mathbf{u}') = \mathbf{0}$$

where $\mathcal{E}$ denotes an ensemble average. The mean current $\mathbf{u}_o(\mathbf{x})$ can be spatially dependent but is temporally constant,

Fig. 2. Basin geometry (top panel) showing the location ξ_j of each within-bay station (solid circles), flux station (open circles), the open boundary, and a number of dimensions. Survey timing (bottom panel) showing two within-bay surveys centred at T_1 and T_2, flux surveys at times T_{fm}, and the time intervals ΔT and Δt_m. The large circles show the details of a within-bay and a flux survey and define the time lags τ_j and δt_m.



while the mean plankton concentration $c_o(\mathbf{x},t)$ can vary with both space and time, as will be seen shortly.

This decomposition into mean and fluctuating parts is not trivial and determines the scope of the problem considered. Full justification requires a gap between "slow" and "fast" variations in the space–time domain. In the present case, the experiment's duration ΔT provides a ready demarkation: variability with periods long relative to ΔT is lumped into the "mean" whereas the remaining variability is included in the fluctuations. Since experiments typically last from 1 to 3 weeks (i.e., the time between surveys), this means that the variability in circulation induced by storms and mesoscale eddies is captured in the fluctuating velocity field $\mathbf{u}'$, while the mean $\mathbf{u}_o$ includes the true mean plus any seasonally varying flow. In practice, the ensemble average for currents would be computed as a time average.

Unlike $\mathbf{u}_o$, the mean plankton field c_o varies on the short time scale as well as the long because of mortality. Similarly, the magnitude of the fluctuations c' must decay because they are bounded by the mean concentration ($c = c_o + c'$ must be positive). To explicitly account for this decay, we redefine

$$(7) \qquad c_o(\mathbf{x},t) \to m(t)c_o(\mathbf{x}) \quad c'(\mathbf{x},t) \to m(t)c'(\mathbf{x},t)$$

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Table 1. Glossary of symbols.

A	basin area (m^2)
$C_T = \langle C(T) \rangle$	areal-averaged plankton concentration at time T (no.·m^{-3})
$\hat{C}_T$	realistic estimate of C_T (no.·m^{-3})
$\overline{C}$	time–basin-averaged plankton concentration (no.·m^{-3})
$\hat{C}$	realistic estimate of $\overline{C}$ (no.·m^{-3})
J	number of stations in a full-basin survey
$J' = J^2 / \sum_j m^2(\tau_j)$	"effective" number of stations in a full-basin survey
K_H	horizontal turbulent kinematic diffusivity ($m^2 \cdot s^{-1}$)
L_x	basin width (m)
L_y	basin length (m)
N	number of flux surveys
M	number of stations in a flux survey
R_{vc}	zero lag cross-covariance between v' and c' along the open boundary (no.·$m^{-2} \cdot s^{-1}$)
$S_{u,v}(\mathbf{k},\omega)$	velocity wave number – frequency spectrum ($m^4 \cdot s^{-1}$)
$S_{uv}(\mathbf{k},\omega)$	cross-spectrum between u' and v' ($m^4 \cdot s^{-1}$)
$S_c(\mathbf{k},\omega)$	plankton wave number spectrum (no.^{2}·$m^{-4} \cdot s^{-1}$)
$S_{vc}(\mathbf{k},\omega)$	cross-spectrum between v' and c' (no.)
T_{fn}	central time of nth flux survey (s)
T_1, T_2 ($T_1 = 0$)	central times of full-basin surveys (s)
Z	biological mortality (s^{-1})
Z_e	effective mortality (s^{-1})
Z_{diff}	mortality contribution due to diffusion (s^{-1})
Z_{flux}	mortality contribution due to advection (s^{-1})
Z_{num}	apparent mortality contribution due to within-basin population change (s^{-1})
$\hat{Z}_{flux}$, $\hat{Z}_{num}$	realistic estimate of Z_{flux}, Z_{num} (s^{-1})
$c(x,y,t) = m(t)(c_o + c')$	plankton concentration (no.·m^{-3})
$c_o(x,y)$	mean plankton concentration (no.·m^{-3})
$c'(x,y,t)$	fluctuating component of plankton concentration (no.·m^{-3})
$\mathbf{k} = k_x, k_y$	wave number in the x,y direction (m^{-1})
$m(t) = e^{-Z_e t}$	mortality function
t	time (s)
t_{mn}	central time of mth station in nth flux survey (s)
$\mathbf{u} = \mathbf{u}_o + \mathbf{u}'$	horizontal velocity (m·s^{-1})
$\mathbf{u}_o(x,y) = (u_o, v_o)$	mean horizontal velocity (m·s^{-1})
$\mathbf{u}'(x,y,t) = (u', v')$	fluctuating component of horizontal velocity (m·s^{-1})
$\mathbf{x} = (x,y)$	horizontal coordinate (m)
Δ	diffusive plankton flux across open boundary (no.·$m^{-2} \cdot s^{-1}$)
$\Delta T = T_2 - T_1$	time interval between plankton censuses (s)
Δt_n, $n = 1, \ldots, N$	time interval to which nth flux survey applies (s)
Δl_m	width of mth subinterval (m)
$\Psi = \Psi_n$, $n = 0, \ldots, 3$	true advective plankton flux across open boundary (no.·$m^{-2} \cdot s^{-1}$)
$\hat{\Psi} = \hat{\Psi}_n$, $n = 0, \ldots, 8$	realistic estimate of Ψ (no.·$m^{-2} \cdot s^{-1}$)
$\beta = \hat{C}_o / \overline{C}_o$	ratio of realistic estimate of the overall plankton concentration to true value
$\delta t_m = t_{mn} - T_{fn}$	time lag of mth station relative to survey central time (s)
ϵ_c	field sampling error in plankton measurement (no.·m^{-3})
$\epsilon_{u,v}$	field sampling error in velocity measurement (m·s^{-1})

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Table 1 (*concluded*).

ξ_j	vector space lag of jth basin station relative to basin centre (m)
σ_c^2	variance of plankton measurement error (no.^{2}·m^{-6})
σ_u^2	variance of velocity measurement error (m^2·s^{-2})
$\tau_j = t_j - T_{1,2}$	time of jth basin station relative to survey central time (s)
ω	frequency (s^{-1})
$\mathcal{E}(\cdot)$	ensemble average
$\langle(\cdot)\rangle$	basin-averaged quantity
$\nabla = (\partial/\partial x, \partial/\partial y)$	horizontal gradient operator (m^{-1})

where the nondimensional, deterministic function $m(t) = e^{-Z_e t}$ depends on the effective mortality Z_e to be defined shortly. Strictly speaking, $c_o(\mathbf{x})$ cannot be estimated prior to the determination of Z_e. Afterwards, it could be computed as a weighted time average.

The fluctuating fields $\mathbf{u}'$ and c' are assumed to be random functions, stationary in time and space. In order to explore the importance of variability at various length and time scales, we adopt a Fourier approach and work in terms of the respective spatial–temporal auto- and cross-spectra of the fluctuations, denoted here in shorthand fashion as $S_{u,v,c}(\mathbf{k},\omega)$ and $S_{uv,uc,vc}(\mathbf{k},\omega)$, which are functions of the horizontal wave number $\mathbf{k} = (k_x,k_y)$ and frequency ω. Considering $u(\mathbf{x},t)$ as an example, we recall that its autospectrum is defined such that the area under its spectral curve equals its variance (viz. $\mathrm{var}(u) = \int S_u(\mathbf{k},\omega)\,\mathrm{d}\mathbf{k}\mathrm{d}\omega$). The elements $S_u(\mathbf{k},\omega)\,\mathrm{d}\mathbf{k}\mathrm{d}\omega$ give the contribution to the variance at cross-channel length scale $2\pi/k_x$, along-channel length scale $2\pi/k_y$, and time scale $2\pi/\omega$. Similarly, the area under the cross-spectral curves gives the covariance between two variables. The Wiener–Khinchine theorem (e.g., Priestley 1981) states that the autocovariance (e.g., $R_u(\boldsymbol{\xi},\tau) = \mathcal{E}[u(\mathbf{x},t)u(\mathbf{x}+\boldsymbol{\xi},t+\tau)]$) and autospectrum are Fourier transform pairs:

$$(8)\qquad R_u(\boldsymbol{\xi},\tau) = \int S_u(\mathbf{k},\omega)e^{i(\mathbf{k}\cdot\boldsymbol{\xi}-\omega\tau)}\,\mathrm{d}\mathbf{k}\mathrm{d}\omega.$$

An analogous equation holds for the cross-covariance and cross-spectrum. For later convenience, we allow $\mathbf{k}$ and ω to vary over negative as well as positive values, although the negative values denote the same length or time scales as their positive counterparts. This choice will be transparent to the reader.

Since the plankton field is assumed to be passive, its spectrum is not independent of the velocity field. In particular, divergence in the horizontal velocity directly modifies plankton concentration, as can be seen by expanding the flux term in eq. 1, viz., $\nabla\cdot(\mathbf{u}c) = \mathbf{u}\cdot\nabla(c) + c\nabla\cdot\mathbf{u}$. The first term denotes the transport of plankton from one point to another, while the second represents the effect of velocity divergence. Where currents converge ($\nabla\cdot\mathbf{u} < 0$), plankton concentration increases and vice versa. (Even in converging currents, we assume that the plankton are above maximum tow depth.) The horizontal velocity field is not a priori assumed to be nondivergent and consequently is ageostrophically balanced, as is appropriate to an actively wind-forced upper layer. This important aspect of our approach differs from a number of studies of the spatial structure of passive tracers including those of the evolution of phytoplankton and zooplankton patchiness (e.g., Kierstead and Slobodkin 1953; Bennett and Denman 1985). Fluctuations in the divergence field cause fluctuations in local plankton concentration that ultimately lead to variability in estimates of plankton abundance made with realistic, spatially coarse, and nonsynoptic sampling schemes.

Substitution of eqs. 4, 5, and 7 into the ensemble average of eq. 2 furnishes a definition for the effective mortality:

$$(9)\qquad Z_e = Z + \frac{1}{A\langle c_o\rangle}\int_{L_x}[v_o c_o + \mathcal{E}(v'c')]\,\mathrm{d}x - \frac{K_H}{A\langle c_o\rangle}\int_{L_x}\frac{\partial c_o}{\partial y}\,\mathrm{d}x.$$

The effective mortality depends on the total loss of plankton from the system and equals the the true biological mortality plus ensemble mean net losses due to (*i*) advection of the mean plankton field by the mean current, (*ii*) the residual advection of plankton fluctuations due to correlation between the fluctuating fields, and (*iii*) diffusion. Z_e is a deterministic system parameter and does not vary within ensembles.

Initially, the total number of plankton within the basin is fixed so that $\langle c'(t=0)\rangle = 0$. For a closed basin, this condition would hold for all time; for one with an open boundary, advection and diffusion break the constraint, and the number of plankton in the basin at any time becomes a random function. If $\mathcal{N} = A\langle c(t)\rangle$ is the total number of plankton in the basin at any time, then:

$$(10)\qquad \frac{\mathrm{d}}{\mathrm{d}t}\mathcal{N} = -Z_e\mathcal{N} + m(t)A\frac{\mathrm{d}}{\mathrm{d}t}\langle c'\rangle$$

so that

$$(11)\qquad \frac{\mathrm{d}}{\mathrm{d}t}\mathcal{E}(\mathcal{N}) = -Z_e\mathcal{E}(\mathcal{N})$$

and the effective mortality is seen as the rate at which the ensemble mean population decays. Changes in $\langle c'\rangle$ are due to variations in the advective and diffusive fluxes across the open boundary, so that variations in the total number of plankton within a basin depend solely on variations in transport.

Although eqs. 1 and 2 refer to semi-enclosed basins, they apply equally as well to a patch circumscribed by an open boundary and moving with a possibly time-dependent velocity $\mathbf{u}_{\mathrm{patch}}$, provided that a Galiean transform is made such

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that the coordinate system drifts with the patch velocity. Consequently, all the analyses presented in this paper may be easily modified for patch studies. We note, however, that the ratio of patch perimeter to patch area is significantly larger than the corresponding ratio for a semienclosed basin. Thus, effects associated with advective and diffusive fluxes should be that much more important in patch studies. We also note that the assumptions of stationarity of the velocity and plankton field could break down if the patch drifted from one oceanographic regime into another.

Estimating mortality

Instantaneous mortality is estimated by computing the relative change in abundance between a pair of plankton censuses separated by a time interval ΔT. Without error, the true biological mortality is obtained by integrating eq. 2 over time giving

$$(12)\quad Z = -\frac{1}{\bar{C}\Delta T}(C_{T_2} - C_{T_1}) - \frac{L_x}{A\bar{C}}\Psi - \frac{L_x}{A\bar{C}}\Delta$$

$$(13)\quad = Z_{\text{num}} - Z_{\text{flux}} - Z_{\text{diff}}$$

where

$$(14)\quad C_T = \langle c(T)\rangle = m(T)\int_A [c_o(x,y) + c'(x,y,T)]\,\mathrm{d}x\mathrm{d}y$$

$$= C_{oT} + C'_T$$

is the basin-averaged plankton concentration at time T,

$$(15)\quad \bar{C} = \frac{1}{\Delta T}\int_{\Delta T}\langle c(t)\rangle \mathrm{d}t = \frac{1}{A\Delta T}\int_{A\Delta T} m(t)(c_o + c')\,\mathrm{d}x\mathrm{d}y\mathrm{d}t$$

$$= \bar{C}_o + \overline{C'}$$

is the basin mean plankton concentration averaged over the experiment's duration ΔT,

$$(16)\quad \Psi = \frac{1}{L_x\Delta T}\int_{L_x\Delta T} m(t)[v_0(x,L_y) + v'(x,L_y,t)]$$

$$\times [c_o(x,L_y) + c'(x,L_y,t)]\,\mathrm{d}x\mathrm{d}t$$

is the mean advective flux across the open boundary, and

$$(17)\quad \Delta = -\frac{K_H}{L_x\Delta T}\int_{L_x\Delta T} m(t)\frac{\partial}{\partial y}[c_o(x,L_y) + c'(x,L_y,t)]\,\mathrm{d}x\mathrm{d}t$$

is the mean diffusive flux of plankton out of the region. (Note that population measures are uppercase whereas plankton concentrations at any given point or time are lowercase.)

The biological mortality Z decomposes into three terms, Z_{num}, Z_{flux}, and Z_{diff}, respectively, expressing the change in the number of plankton within the basin, the advective flux out of the basin, and diffusion out of the basin. Most mortality estimates are based on Z_{num}, which we term the "apparent mortality". Z_{flux} and Z_{diff} are defined so they are positive for a respective net transport of particles out of the basin. Since $Z_{\text{num}} = Z + Z_{\text{flux}} + Z_{\text{diff}}$, an advective or diffusive loss biases the apparent mortality high relative to the true mortality. It is important to realize that the three mortality terms vary naturally from experiment to experiment in consonance with variations in the environment. That is, estimates of the individual mortalities Z_{num}, Z_{flux}, and Z_{diff} vary even in the absence of measurement error. The true mortality $Z = (Z_{\text{num}} - Z_{\text{flux}} - Z_{\text{diff}})$ does not vary. It is shown in the Appendix that $\mathcal{E}(Z_{\text{num}}) \approx Z_e$ so that the the average value of apparent mortality is the effective, not the true, mortality Z_e. In effect, each estimate of Z_{num} is a realization of Z_e.

Realistic estimators

In practice, a two-point estimate of Z depends on estimates of the following five variables: the spatially averaged abundance C_T at the endpoints $t = T_1 = 0$ and $T_2 = \Delta T$, the space–time averaged plankton concentration $\bar{C}$, and the fluxes Ψ and Δ The error in estimating Z thus depends on the accumulated errors in estimates of these variables that arise from (*i*) experimental sampling variability due to the imperfect nature of nets and current meters, (*ii*) the design of the sampling grid, and (*iii*) the violation of assumptions made to this point. Of these factors, we have significant control only over the second. In the following analysis, we assess the effects of sampling strategy by examining the properties of realistic but imperfect estimators of mortality, flux, and population. In so doing, we will isolate the effects of the inherent space and time variations in the current and plankton fields. For the remainder of the discussion, diffusion is ignored because it is generally much less important than advection (Pepin et al. 1995) and cannot be reliably estimated from the data collected in typical mortality experiments. Of these five variables, two are fundamental, the basin population C_T and the advective flux Ψ, and much of the ensuing analysis focusses on them.

Realistic mortality estimators are defined analogously to eq. 12 but using realistic estimators for C_T, Ψ, and $\bar{C}$. However, since the form for Z_{num} in eq. 12 is biased in practice, we use the alternative, unbiased form in the remaining analysis for its realistic estimate:

$$(18)\quad \hat{Z}_{\text{num}} = -\frac{1}{\Delta T}(\ln\hat{C}_{T_2} - \ln\hat{C}_{T_1}).$$

For the flux mortality estimate, we have simply

$$(19)\quad \hat{Z}_{\text{flux}} = -\frac{L_x}{A\hat{C}}\hat{\Psi}.$$

The fundamental estimators, $\hat{C}_T$ and $\hat{\Psi}$, are specified in terms of discrete sampling plans (Fig. 2). Each full-basin survey is assumed to be carried out in identical fashion: a set of J stations is occupied at points $\{\mathbf{x}_j, j = 1,..., J\}$ with fixed between-station time lags τ_j. (If t_j is the occupation time of the jth station, then $\tau_j = t_j - T$, where T is the central time of the survey. Similarly, a set of spatial separations is defined relative to the basin centre, $\xi_j = (x_j - L_x/2, y_j - L_y/2)$.) The estimator for C_T is

$$(20)\quad \hat{C}_T = \frac{1}{J}\sum_j m(t_j)[c_o(\mathbf{x}_j) + c'(\mathbf{x}_j,t_j) + \epsilon_{cj}]$$

$$= m(T)\frac{1}{J}\sum_j m(\tau_j)[c_{oj} + c'_j + \epsilon_{cj}]$$

$$= \hat{C}_{oT} + \hat{C}'_T + \frac{m(T)}{J}\sum_j m(\tau_j)\epsilon_{cj}$$

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where $m(t_j)\epsilon_c$ is the measurement error in the jth plankton tow and will be specified more explicitly shortly. Errors in estimates of C_T thus arise from two sources: the sampling plan and experimental error.

Estimation of the flux is slightly more complicated (Fig. 2). We assume that N flux surveys are carried out between the two full-basin surveys, that M plankton tows and current measurements are made during each transect, and that each survey is executed in identical fashion. Specifically, the same locations $\mathbf{x}_m = (x_m, L_y)$ are occupied with identical time lags δt_m. (If t_{mn} is the occupation time of the mth station during the nth flux survey, then $\delta t_m = t_{mn} - T_{fn}$, where T_{fn} is the central time of the survey.) Corresponding to each station, we define a length interval Δl_m such that $\sum_m^M \Delta l_m = L_x$, and corresponding to each transect, we define a time interval Δt_n such that $\sum_n^N \Delta t_n = \Delta T$. The advective flux estimate is

$$(21)\quad \hat{\Psi} = \sum_m^M \sum_n^N \left(\frac{\Delta l_m}{L_x}\right)\left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn})[v_{om} + v'_{nm} + \epsilon_{vmn}] \times [c_{om} + c'_{nm} + \epsilon_{cmn}]$$

where ϵ_v is the observational error in a current measurement. (A variety of choices for Δt_n are possible, and the condition that they sum to ΔT may not be desirable depending on the number and timing of the flux surveys. Alternatives are easily accommodated.)

Measurement error in both the plankton samples and currents is modelled as additive, zero-mean white noise such that

$$(22)\quad \mathcal{E}(\epsilon_c) = \mathcal{E}(\epsilon_{u,v}) = \mathcal{E}(\epsilon_u \epsilon_v) = 0$$

$$(23)\quad \mathcal{E}(\epsilon_{ci}\epsilon_{cj}, \epsilon_{ui}\epsilon_{uj}, \epsilon_{vi}\epsilon_{vj}) = (\sigma_c^2, \sigma_u^2, \sigma_v^2)\delta_{ij}$$

where i and j denote individual observations and $\delta_{ij} = 1$ if $i = j$ and 0 otherwise; $m^2(t)\sigma_c^2$ and σ_v^2 are the respective measurement error variances. The assumed time dependence of the tow error variance allows it to be proportional to the mean plankton concentration in accord with observations (Cyr et al. 1992; Pepin and Shears 1997). This is not a restrictive assumption, however, and can easily be relaxed.

In terms of mean and fluctuating quantities, $\hat{\Psi}$ becomes

$$(24)\quad \hat{\Psi} = \hat{\Psi}_0 + [\hat{\Psi}_1 + \hat{\Psi}_2 + \hat{\Psi}_3] + [\hat{\Psi}_4^\epsilon + \hat{\Psi}_5^\epsilon + \hat{\Psi}_6^\epsilon + \hat{\Psi}_7^\epsilon + \hat{\Psi}_8^\epsilon]$$

$$= \sum_m \left(\frac{\Delta l_m}{L_x}\right) v_{om} c_{om} \sum_n \left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn}) \quad \text{(0) transport of the mean plankton field by the mean flow}$$

$$+ \sum_m \left(\frac{\Delta l_m}{L_x}\right) v_{om} \sum_n \left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn}) c'_{mn} \quad \text{(1) transport of the fluctuating field by the mean flow}$$

$$+ \sum_m \left(\frac{\Delta l_m}{L_x}\right) c_{om} \sum_n \left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn}) v'_{mn} \quad \text{(2) transport of the mean field by velocity fluctuations}$$

$$+ \sum_m \sum_n \left(\frac{\Delta l_m}{L_x}\right)\left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn}) v'_{mn}\, c'_{mn} \quad \text{(3) transport of plankton fluctuations by the fluctuating current}$$

$$+ \sum_m \left(\frac{\Delta l_m}{L_x}\right) v_{om} \sum_n \left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn}) \epsilon_{cnm} \quad \text{(4) apparent plankton transport by } v_o \text{ due to plankton sampling error}$$

$$+ \sum_m \left(\frac{\Delta l_m}{L_x}\right) \epsilon_{vmn} c_{om} \sum_n \left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn}) \quad \text{(5) apparent transport of the mean field due to current measurement error}$$

$$+ \sum_m \sum_n \left(\frac{\Delta l_m}{L_x}\right)\left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn}) v'_{mn}\, \epsilon_{cmn} \quad \text{(6) apparent transport by } v' \text{ due to plankton measurement error}$$

$$+ \sum_m \sum_n \left(\frac{\Delta l_m}{L_x}\right)\left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn}) \epsilon_{vmn} c'_{mn} \quad \text{(7) apparent transport of } c' \text{ due to current measurement error}$$

$$+ \sum_m \sum_n \left(\frac{\Delta l_m}{L_x}\right)\left(\frac{\Delta t_n}{\Delta T}\right) m(t_{mn}) \epsilon_{vmn} \epsilon_{cmn} \quad \text{(8) apparent transport due to combined sampling errors.}$$

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The last five terms express measurement error as denoted by the superscript ϵ, while the first four terms have their counterparts in the decomposition of the true flux:

$$(25)\quad \Psi = \frac{1}{L_x \Delta T}\int_{L_y} m(t)[v_o c_o + v_o c' + v' c_o + v' c']\,\mathrm{d}x\mathrm{d}t = \Psi_0 + \Psi_1 + \Psi_2 + \Psi_3.$$

In fact, $\hat{\Psi}$ can be rewritten conveniently in terms of the true value, the error due to discretisation (i.e., coarse sampling), and measurement error as

$$(26)\quad \hat{\Psi} = \Psi + \sum_{j=0}^{3}(\hat{\Psi}_j - \Psi_j) + \sum_{j=4}^{8}\hat{\Psi}_j^{\epsilon}.$$

The estimator for the survey mean full-basin concentration $\overline{C}$ is defined as the average of the estimates for the two full-basin surveys and can be written in obvious fashion as

$$(27)\quad \hat{C} = \frac{1}{2}(\hat{C}_{T_1} + \hat{C}_{T_2}) = \hat{C}_o + \hat{C}' + \frac{1}{2J}\left\{\sum_{j_1} m(\tau_{j_1})\epsilon_{cj_1} + m(\Delta T)\sum_{j_2} m(\tau_{j_2})\epsilon_{cj_2}\right\}.$$

The sums over j_1 and j_2 refer to the first and second basin surveys, respectively.

Statistics of mortality estimates

In this section, we derive expressions for the statistics of the basic population estimators $\hat{C}_T$ and $\hat{\Psi}$, the derived estimator $\hat{C}$, and the mortality estimators $\hat{Z}_{\text{num}}$ and $\hat{Z}_{\text{flux}}$. We use a variety of statistical measures including $\text{bias}(X) = \mathcal{E}(X - X_{\text{true}})$, $\text{var}(X) = \mathcal{E}([X - \mathcal{E}(X)]^2)$, and $\text{var}(X - X_{\text{true}}) = \mathcal{E}([X - X_{\text{true}} - \mathcal{E}(X - X_{\text{true}})]^2)$. The last is related to the mean square error by $\text{mse}(X) = \mathcal{E}([X - X_{\text{true}}]^2) = \text{var}(X - X_{\text{true}}) + \text{bias}^2(X)$.

Population and flux estimators

Variance

We earlier emphasized that both the within-basin plankton concentration as well as the flux of plankton out of the basin are subject to natural environmental variability. In this section, we give equations describing how the variability in both the true and estimated population and flux measures is distributed among space and time scales. Using the methods outlined in the Appendix, we find that

$$(28)\quad \text{var}(C_T) = m^2(T)\int S_c(\mathbf{k},\omega)\left|H(\mathbf{k})\right|^2 \mathrm{d}\mathbf{k}\mathrm{d}\omega$$

$$(29)\quad \text{var}(\hat{C}_T) = m^2(T)\left[\int S_c(\mathbf{k},\omega)\left|\hat{H}(\mathbf{k},\omega)\right|^2 \mathrm{d}\mathbf{k}\mathrm{d}\omega + \frac{\sigma_c^2}{J'}\right]$$

where $J' = J^2/\sum_j m^2(\tau_j) \approx J$ is the "effective" number of stations. (Over the duration of a survey, $m(\tau_j) \approx 1$, and the sum over its square is almost equal to J.) $H(\mathbf{k})$ is a real-valued, deterministic, nondimensional function:

$$(30)\quad H(\mathbf{k}) = \frac{\mathrm{e}^{i(k_x L_x/2 + k_y L_y/2)}}{A}\int_A \mathrm{e}^{i(k_x x + k_y y)}\,\mathrm{d}x\mathrm{d}y = \frac{\sin(k_x L_x/2)}{k_x L_x/2}\,\frac{\sin(k_y L_y/2)}{k_y L_y/2}$$

while $\hat{H}(\mathbf{k},\omega)$ is complex valued, deterministic, and nondimensional and is determined by the sampling plan

$$(31)\quad \hat{H}(\mathbf{k},\omega) = \frac{1}{J}\sum_j m(\tau_j)\mathrm{e}^{i(\mathbf{k}\cdot\xi_j - \omega\tau_j)} \approx \frac{1}{J}\sum_j \mathrm{e}^{i(\mathbf{k}\cdot\xi_j - \omega\tau_j)}.$$

The phase factor $\mathrm{e}^{i(k_x L_x + k_y L_y)/2}$ in eq. 30 can be ignored as H always appears as its absolute value squared $\left|H(\mathbf{k})\right|^2$.

Equations 28 and 29 partition the estimator variance according to the space and time scales of natural fluctuations in plankton concentration. Regions in wave number – frequency space where $H(\mathbf{k})$ and $S_c(\mathbf{k},\omega)$ significantly overlap are those regions that contribute to the natural variability of C_T. Additional regions in which $\hat{H}(\mathbf{k},\omega)$ and $S_c(\mathbf{k},\omega)$ overlap are regions that generate undesirable variability in the realistic estimates $\hat{C}_T$. That is, a sampling plan that fails to damp the energetic space and time scales of the plankton population will generate population estimates whose variance is greater than natural levels. These, and similar relationships that follow, form the core result of this paper. Correspondingly, understanding the functions H and $\hat{H}$ is fundamental to understanding the effects of environmental variability on population estimation. The more closely $\hat{H}$ resembles H, the better the sampling plan:

$$(32)\quad \text{var}(\hat{C}_T - C_T) = m^2(T)\left\{\int S_c(\mathbf{k},\omega)\left|\hat{H}(\mathbf{k},\omega) - H(\mathbf{k})\right|^2 \mathrm{d}\mathbf{k}\mathrm{d}\omega + \frac{\sigma_c^2}{J'}\right\}.$$

Although their graphs may prove complicated and full of detail, both H and $\hat{H}$ are easy to compute. They require a knowledge only of the times and locations of the sampling stations and a guess at the effective mortality Z_e.

H is the product of two frequency-independent, damped sinusoids with a maximum value of 1 at the origin ($k_x, k_y = 0$) and successive zeros at $2\pi/k_x = L_x/n$ or $2\pi/k_y = (L_y/n)$, $n = 1, 2, \ldots$ (i.e., at length scales that are simple fractions of the basin width or length). At length scales less than the basin extent, H^2 has a maximum value less than 0.05. Thus, only fluctuations with length scales in excess of L_x or L_y contribute significantly to the variance of the true values, albeit at all frequencies. In semi-enclosed basins, this variance would be small. However, such variability could assume more significance for patch studies in which current fluctuations at length scales greater than the patch diameter would be more likely to be energetic.

$\hat{H}(\mathbf{k},\omega)$ is a discrete analogue of H and carries a complete description of the sampling array in wave number – frequency space. Because the array is discrete in space and time, $\hat{H}$ is not damped like H but is periodic in both wave number and frequency (e.g., Oppenheim and Schafer 1975). Consequently, $\hat{H}$ is similar to H near the origin ($k_x, k_y, \omega = 0$) but displays a much more complicated structure elsewhere. In particular, it exhibits periodicities corresponding

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to all of the sums and differences of the space and time lags ξ_j and τ_j. Note that $\hat{H}$ depends on the space and time separations but does not explicitly depend on either the basin width or length or the survey duration. The longest scales damped by the sampling scheme are limited by the largest space and time lags.

Recalling that the spectrum of a process is defined such that its variance is the area under its spectral curve, we see that $\left|\hat{H}(\mathbf{k}, \omega)\right|^2 S_c(\mathbf{k}, \omega)$ forms the spectrum for that part of $\hat{C}_T$ due to environmental variability. From this viewpoint, H and $\hat{H}$ act as filters. In particular, $\left|H(\mathbf{k})\right|^2$ is a low-pass filter that admits variability only at long scales, while $\left|\hat{H}(\mathbf{k}, \omega)\right|^2$ is an approximation to $\left|H(\mathbf{k})\right|^2$ that generally resembles it at long space and time scales. The second term in eq. 29, which is due solely to measurement error, may be considered as a "white noise" component constant across the full $\hat{C}_T$ spectrum.

The variance of the time-averaged full-basin population estimator is given by a similar expression:

$$\text{(33)}\quad \text{var}(\hat{C}) = \frac{1}{4}\int S_c(\mathbf{k}, \omega)[1 + 2m(\Delta T)\cos(\omega\Delta T) + m^2(\Delta T)]\left|\hat{H}(\mathbf{k}, \omega)\right|^2 \mathrm{d}\mathbf{k}\mathrm{d}\omega + \frac{1+m^2(\Delta T)}{4J'}\sigma_c^2$$

and has essentially the same interpretation as eq. 29 except for its dependence on experiment duration. The term $2m(\Delta T)\cos(\omega\Delta T)$ expresses effect of the correlation between the first and second surveys. If the surveys were uncorrelated, it would vanish, and the first term in eq. 33 would be $[1 + m^2(\Delta T)]\text{var}(\hat{C}_T)/4 < \text{var}(\hat{C}_T)/2$. To see the influence of this term, consider the part of the plankton field that varies naturally with some frequency ω_a. The variance in the estimated abundance of that frequency component over the full survey is minimized if the two surveys are conducted when the fluctuations are 180° of of phase. In effect, variations sampled during the first survey are cancelled by those sampled during the second. Since the concentration decreases between surveys, the cancellation is only partial as measured by $m(\Delta T)$. It thus appears that if a system is characterized by some energetic frequency, one could choose ΔT to minimize $\text{var}(\hat{C})$. However, we will soon show that such a choice would maximize the error in $\hat{Z}_{\text{num}}$, so the choice is not clear-cut.

The variance of the flux estimator has a more complicated form commensurate with the number of components that contribute to it. It can be shown that

$$\text{(34)}\quad \text{var}(\hat{\Psi} - \Psi) = \sum_{j=1}^{3}\text{var}(\hat{\Psi}_j - \Psi_j) + 2\mathscr{E}[(\hat{\Psi}_1 - \Psi_1)(\hat{\Psi}_2 - \Psi_2)] + \sum_{j=4}^{8}\text{var}(\hat{\Psi}_j^{\mathrm{c}})$$

if third-order correlations are ignored (and which vanish if the fluctuations are Gaussian distributed). Thus, with one exception, the constituent parts of $\hat{\Psi}$ and Ψ are "orthogonal" and the total variance is the sum of the constituent variances. The variance in $\hat{\Psi}$ has a form exactly analogous to eq. 29:

$$\begin{aligned}\text{(35)}\quad \text{var}(\hat{\Psi}) \approx\ & \int S_v(\mathbf{k}, \omega)\left|\hat{F}_C(k_x, \omega)\right|^2 \hat{M}(\omega)\mathrm{d}\mathbf{k}\mathrm{d}\omega && [\text{var}(\hat{\Psi}_1)]\\ &+ \int S_c(\mathbf{k}, \omega)\left|\hat{F}_V(k_x, \omega)\hat{M}(\omega)\right|^2 \mathrm{d}\mathbf{k}\mathrm{d}\omega && [\text{var}(\hat{\Psi}_2)]\\ &+ 2\int S_{vc}(\mathbf{k}, \omega)\hat{F}_C^*(k_x, \omega)\hat{F}_V(k_x, \omega)\left|\hat{M}(\omega)\right|^2 \mathrm{d}\mathbf{k}\mathrm{d}\omega && \mathscr{E}[(\hat{\Psi}_1\hat{\Psi}_2)]\\ &+ \left(\int [S_c(\mathbf{k}, \omega)S_v(\mathbf{k}', \omega') + S_{vc}(\mathbf{k}, \omega)S_{vc}(\mathbf{k}', \omega')]\left|\hat{Q}(k_x - k_x', \omega - \omega')\hat{M}(\omega - \omega')\right|^2 \mathrm{d}\mathbf{k}\mathrm{d}\mathbf{k}'\mathrm{d}\omega\mathrm{d}\omega'\right) && [\text{var}(\hat{\Psi}_3)]\\ &+ \left(\sum_m\left(\frac{\Delta l_m}{L_x}\right)^2 m^2(\delta t_m)v_{om}^2\right)\left(\sum_n\left(\frac{\Delta t_n}{\Delta T}\right)^2 m^2(T_{fn})\right)\sigma_c^2 && [\text{var}(\hat{\Psi}_4^{\mathrm{c}})]\\ &+ \left(\sum_m\left(\frac{\Delta l_m}{L_x}\right)m(\delta t_m)c_{vm}\right)^2 \hat{M}^2(0)\,\sigma_v^2 && [\text{var}(\hat{\Psi}_5^{\mathrm{c}})]\end{aligned}$$

Here:

$$\text{(36)}\quad \hat{F}_C(k_x, \omega) = \sum_m\left(\frac{\Delta l_m}{L_x}\right)m(\delta t_m)c_{om}\mathrm{e}^{ik_x x_m}\mathrm{e}^{-i\omega\delta t_m}$$

$$\text{(37)}\quad \hat{F}_V(k_x, \omega) = \sum_m\left(\frac{\Delta l_m}{L_x}\right)m(\delta t_m)v_{om}\mathrm{e}^{ik_x x_m}\mathrm{e}^{-i\omega\delta t_m}$$

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$$(38)\quad \hat{Q}(k_x,\omega)=\sum_m\left(\frac{\Delta l_m}{L_x}\right)m(\delta t_m)e^{i(k_x x_m-\omega\delta t_m)}$$

$$(39)\quad \hat{M}(\omega)=\sum_n\left(\frac{\Delta t_n}{\Delta T}\right)m(T_{fn})e^{-i\omega T_{fn}}$$

To obtain eq. 35, we ignored all correlations higher than second order in the fluctuations, except for the fourth-order term $\mathrm{var}(\hat{\Psi}_3)$, which we approximated by assuming Gaussian statistics for c' and v' and expanding as a sum of second-order terms (Isserlis 1918). The asterisk in eq. 35 denotes the complex conjugate. In fact, S_{vc}, $\hat{F}_C$, $\hat{F}_V$, $\hat{Q}$, and $\hat{M}$ are all complex-valued functions, but the device of allowing k_x, k_y, and ω to vary over negative as well as positive values ensures that all the terms in eq. 35 are real.

The terms in eq. 35 represent variability due to (*i*) undersampling open boundary velocity fluctuations that transport the mean plankton field, (*ii*) undersampling open boundary fluctuations in plankton concentration that are transported by the mean current, (*iii*) undersampling the interaction between the correlated part of the fluctuations and the mean plankton and velocity fields, (*iv*) undersampling the joint fluctuations in plankton and currents, (*v*) measurement error of the plankton field transported by the mean flow, and (*vi*) measurement error of the fluctuating velocity field that leads to an apparent transport of mean plankton concentration. All of the mean and fluctuating velocity and plankton fields contribute to the variance.

$\hat{F}_C(k_x,\omega)$ and $\hat{F}_V(k_x,\omega)$ are discrete Fourier transforms of the mean plankton and velocity fields and thus are largest on the spatial scales on which these fields vary. Since these scales are generally long, the first three terms in eq. 35 tend to be important only if the fluctuating fields also vary on long scales. Similarly, $\hat{M}(\omega)$ is the Fourier transform of the mortality function and is peaked about zero frequency with its largest values at time scales greater than ΔT. Consequently, the first three terms in eq. 35 tend to select large time – large space scale variability in c' and v'. Interpretation of the fourth term is somewhat more complicated. However, since $\left|\hat{Q}(k_x)\hat{M}(\omega)\right|^2$ is strongly peaked about the origin $k_x,\omega=0$, it can be approximated by a delta function so that

$$\mathrm{Term\ 4}=\mathrm{var}(\hat{\Psi}_3)\approx\int[S_c(\mathbf{k},\omega)S_v(\mathbf{k}',\omega')+S_{vc}(\mathbf{k},\omega)S_{vc}(\mathbf{k}',\omega')]\,d\mathbf{k}d\omega d\mathbf{k}'d\omega'.$$

Thus, in order for $\hat{\Psi}_3$ to generate significant variance, it is necessary that the plankton and velocity fields vary coincidentally on the same length and time scales (which is almost certainly the case). The cross-spectrum S_{vc} is rarely known, however, so it is very difficult to estimate its contribution.

Each term in eq. 35 is evaluated in Helbig and Pepin (1998) using field data, and excellent agreement is obtained with the variance of the observed fluxes.

Bias

Realistic estimators of the within-basin population and the advective flux are biased to some degree by undersampling:

$$(40)\quad \mathrm{bias}(\hat{C}_T)=\frac{m(T)}{J}\sum_j c_o(x_j)m(\tau_j)-C_{oT}=m(T)\left(\frac{\hat{C}_{o1}}{C_{o1}}-1\right)C_{o1}$$

$$(41)\quad \mathrm{bias}(\hat{\Psi})=(\hat{\Psi}_o-\Psi_0)+\mathcal{E}(\hat{\Psi}_3).$$

These biases are principally determined by how well the mean fields are sampled. Although it is not possible to determine either the signs or magnitudes without detailed consideration of the sampling plan, a few general statements are possible. Equation 40 shows that realistic estimates of C_T are probably only slightly biased provided that the number of stations J is sufficiently large and that surveys are not too long (so that $\sum_j m(\tau_j)\approx 1$). Moreover, the fractional bias $(\mathrm{bias}(\hat{C}_T/C_T)$ remains constant with time as the population concentration C_{oT} diminishes. The bias in the flux estimate depends not only on how well the mean fields are sampled, but also on the correlation between the fluctuating fields along the open boundary. In fact, the second term in eq. 41 is proportional to $R_{vc}=\mathcal{E}[v'(x,L_y,t)c'(x,L_y,t)]$, the cross-covariance between v' and c' along the open boundary at zero time and space lag. It can be shown that since $m(t)$ is concave, the proportionality constant is positive for reasonable sampling schemes. Thus, a positive correlation between plankton and currents generally implies a positive contribution to the flux bias.

However, the bias in estimates of the $\overline{C}$,

$$(42)\quad \mathrm{bias}(\hat{\overline{C}})=\left[\frac{Z_e\Delta T/2}{\tanh(Z_e\Delta T/2)}\frac{\hat{C}_{o1}}{C_{o1}}-1\right]\overline{C}_o$$

can be significant depending on the survey duration. Basically, the bias arises from the implicit assumption in a two-point estimate that the population changes linearly with time, not exponentially. Consequently, the time factor $(Z_e\Delta T/2)/\tanh(Z_e\Delta T/2)$ is always greater than 1. For small nondimensional time $Z_e\Delta T$ (corresponding to relatively short between-survey times), it is close to 1 and contributes little to the bias. For long survey times, it increases linearly with $Z_e\Delta T$ and can lead to significant bias. For example, assuming that the spatial factor $\hat{C}_{o1}/C_{o1}$ is approximately 1 and that $Z_e\approx 0.1\cdot\mathrm{day}^{-1}$, we compute fractional biases of 2, 8, and 18% for ΔT = 5, 10, and 15 days, respectively.

Mortality estimators

Derivation of expressions for the statistics of the mortality estimates is complicated by their nonlinear nature (i.e., the random variables appear in logarithms or quotients). We give here expressions for the variance of $\hat{Z}_{\mathrm{num}}$ and $\hat{Z}_{\mathrm{flux}}$ approximated using the methods outlined in the Appendix. A more complete set of expressions is listed in the Appendix.

For $\hat{Z}_{\mathrm{num}}$, we find

$$(43)\quad \mathrm{var}(\hat{Z}_{\mathrm{num}})=\frac{2}{\Delta T^2\hat{C}^2_{oT_1}}\int S_c(\mathbf{k},\omega)[1-\cos(\omega\Delta T)]\times\left|\hat{H}(\mathbf{k},\omega)\right|^2 d\mathbf{k}d\omega+\frac{2}{\Delta T^2\hat{C}^2_{oT_1}}\frac{\sigma_c^2}{J'}.$$

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Equation 43 carries essentially the same interpretation as eq. 29 except for its dependence on the intersurvey time through the frequency-dependent term $f_1(\omega) = 1 - \cos(\omega\Delta T)$. This factor accounts for the relative difference in phase between the beginning and the end of the experiment of each frequency component of the plankton spectrum. It was discussed in conceptual terms by Legendre and Demers (1984) and Taggart and Frank (1990) for the special case in which the environment varies monochromatically with the tides. In its more general setting, it applies to variability on all time scales. Briefly, if the abundance varies with some frequency $\omega_a = 2\pi/T_a$, no extra variance is introduced into the estimate if the system is sampled at the same relative position in the cycle of period T_a (i.e., if $\Delta T = T_a, 2T_a, \ldots$). The worst case occurs when the two samples are taken 180° apart (e.g., at high and low tide).

The relationship for the variance of $\hat{Z}_{\text{flux}}$ is given in symbolic form by

$$(44)\quad \text{var}(\hat{Z}_{\text{flux}}) = \left(\frac{L_x}{A\hat{C}_o}\right)^2 \left\{ \text{var}(\hat{\Psi}) + \frac{1}{\hat{C}_o^2}\hat{\Psi}_o^2 \text{var}(\hat{C}') + \frac{2\hat{\Psi}_o}{\hat{C}_o}[\mathcal{E}(\hat{\Psi}_1\hat{C}' + \hat{\Psi}_2\hat{C}')] + \hat{\Psi}_o^2 \frac{1+m^2(\Delta T)}{J'}\sigma_c^2 \right\}$$

and the reader is referred to eqs. 33, 35, and A13 for appropriate spectral forms of the individual terms. Interpretation of eq. 44 is straightforward: the variance in the flux mortality estimates is due to variance in the flux and population estimates themselves (first two terms) as well as to more complicated interactions between the velocity and plankton fluctuations on the open boundary (third term) and to measurement error (fourth term).

These expressions provide a ready technique for estimating expected error and helping in the design of an "optimal" sampling strategy prior to field work. Given guesses at the velocity and plankton concentration spectra, the leading terms in eqs. 43 and 44 can easily be evaluated numerically to provide insight into the choice of sampling array. These expressions are estimated in Helbig and Pepin (1998) and compare very favourably with a set of numerical simulations.

Discussion

Accurate measurement of mortality (and growth) rates is fundamental to determining the factors that influence the dynamics of the early life stages of fish, which in turn appear to be important to ultimately determining their relative year-class strengths. For example, accurate knowledge of these rates is vital in understanding the the role of predator–prey interactions in regulating egg and plankton survival. However, mortality estimates are highly variable, and simple scale arguments or models indicate that very modest currents can make a contribution to the overall observed mortality that is equal to the biological component one desires to measure. Consequently, it is necessary to attempt to remove the physical component from estimates.

We have developed a framework that shows how the true values and realistic estimates of population measures, physical transports, and various measures of mortality vary with environmental fluctuations. Estimates vary to a greater degree than do true values, as realistic sampling plans cannot accommodate all the variability in the plankton and velocity fields. Our theory partitions the bias and variance of the estimates according to the space and time scales of natural variability of the underlying current and plankton fields. We reiterate that this theory can be extended to Lagrangian experiments that track plankton patches, provided that the coordinate system is transformed to one moving with the mean patch velocity. Since the patch perimeter to area ratio is larger than the corresponding ratio for semienclosed systems, we anticipate natural variability to be of greater importance in open-water systems.

Understanding this theory implies understanding the roles of a set of deterministic functions like $\hat{H}$, $\hat{F}_V$, and $\hat{F}_C$. In the following example, we consider estimates of the within-bay population C_T and the apparent mortality Z_{num}, and in so doing illustrate the role played by $\hat{H}$. That played by the other functions is similar, and discussion of flux-related estimates is deferred to Helbig and Pepin (1998), where a field experiment designed to measure Z_{flux} is critically evaluated within this formalism.

To visualize H and $\hat{H}$ and to test our intuition, we consider the case of what would appear to be an excellent sampling plan for a 20 × 20 km basin. This plan requires 24 h to complete, during which time, 36 stations arrayed in six rows of six stations each are sampled in a rowwise fashion with constant interstation spacings of 3.33 km and 0.68 h (Fig. 3A). Traditional sampling theory for uniform grids (e.g., Blackman and Tukey 1959) states that variability can be resolved on scales extending from the overall size or duration of the array down to two times the interstation spacing (2π/Nyquist wave number or frequency). Variability at larger scales appears as trends in the data, while that at smaller scales is "aliased" (i.e., incorrectly appears at lower frequencies) and corrupts spectral estimates. Thus, based on our earlier considerations, we would expect natural variability in C_T to appear on all time scales and on length scales greater than 20 km and in $\hat{C}_T$ on time scales greater than 24 h and length scales longer than 20 km. We might also expect $\hat{C}_T$ to damp fluctuations with length scales between 2 × 3.33 = 6.66 km and $L = L_x = L_y = 20$ km and time scales between 2 × 0.68 = 1.36 and 24 h. In fact, however, we would be wrong, as the appropriate lower limits are actually 0.68 h and 3.33 km as $|\hat{H}(\mathbf{k}, \omega)|^2$ peaks at time and length scales equal to the sampling intervals, not twice the intervals. The reasons for this are deferred until the end of this section. We thus anticipate $\hat{H}$ to be small only within the the wave number range of 1/20–1/3.3 = 0.05–0.3·km^{-1} and the frequency range of 1/24–1/0.68 = 0.042–1.48·h^{-1} and expect H to be large only at wave numbers less than 0.05·km^{-1}. We also expect $|\hat{H}|^2$ to be roughly periodic at wave numbers of 0.3· km^{-1} and a frequency of 1.48·h^{-1}.

Figure 3 provides several two-dimensional slices of H^2 and $|\hat{H}|^2$ over selected wave number and frequency ranges. As expected, H is small except at length scales greater than L (Fig. 3B). At very low frequency (Fig. 3C), $|\hat{H}|^2$ is symmetric in k_x and k_y and is periodic with a wave number of 0.3·km^{-1} as anticipated. This periodicity is especially noticeable at small wave numbers where copies of the main peak centred at the origin are apparent. The peak width depends

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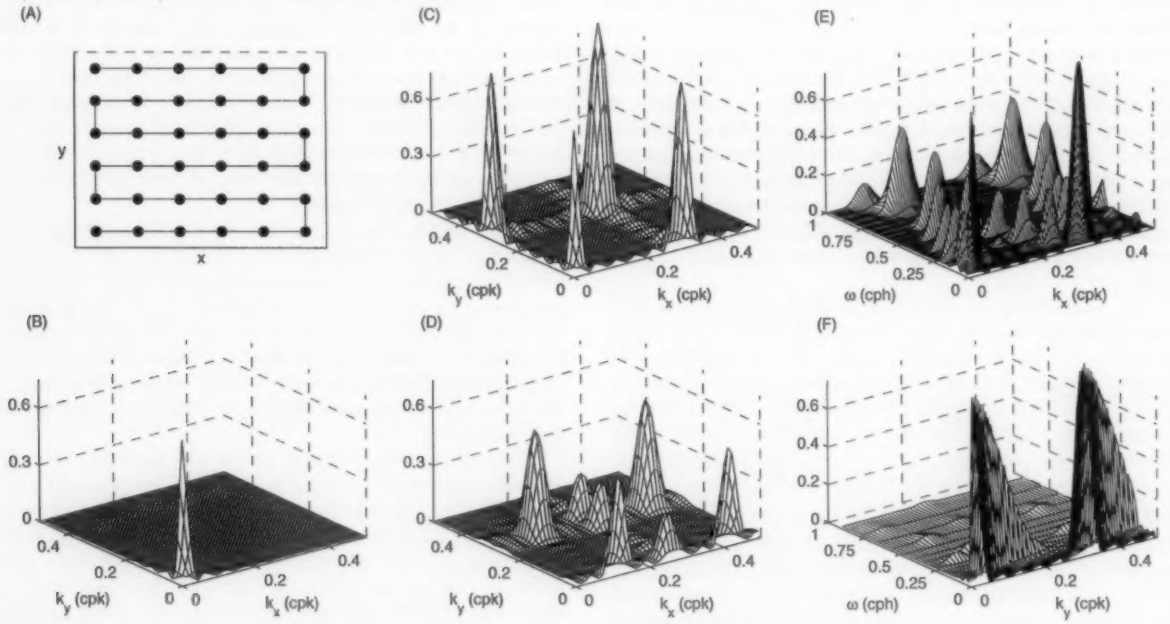
Fig. 3. $\hat{H}$ for a 20 × 20 km basin and a sampling plan of 36 stations arrayed in six lines of six stations each with interstation spacings of 3 km and 40 min. (A) Sampling plan; (B) $\left|\hat{H}(\mathbf{k})\right|^2$; (C) $\left|\hat{H}(\mathbf{k}, \omega = 0)\right|^2$; (D) $\left|\hat{H}(\mathbf{k}, \omega = 1/10\ \text{h})\right|^2$; (E) $\left|\hat{H}(k_x, k_y = 0, \omega)\right|^2$; (F) $\left|\hat{H}(k_x = 0, k_y, \omega)\right|^2$. cpk, cycles·km^{-1}; cph, cycles·h^{-1}.



on L and ΔT with halfwidths of $k/2\pi = 0.433/L$ and $\omega/2\pi = 0.433/T_{\text{survey}}$; the larger the basin or survey time, the narrower the peaks. At higher frequency (Fig. 3D), the pattern is no longer symmetric as a ridge extends along $k_y = 0.3\cdot\text{km}^{-1}$ and the peaks have shifted to larger k_x values corresponding to cross-basin length scales of 8 and 2.4 km. Between scales of 4 and 20 km, $\hat{C}_T$ is immune to along-basin environmental variability, although it is overall more affected than at lower frequencies. The asymmetry originates in the asymmetric character of the sampling plan in which stations separated in the along-basin direction are sampled on average 6 h apart whereas cross-basin stations are only separated by 40 min. At low frequencies, the difference in between-station times is unimportant, as the plankton field is essentially frozen, and $\hat{H}$ is consequently symmetric. At shorter time scales the effect becomes important as between-station times become an appreciable fraction of the time scale.

This asymmetry is more dramatically illustrated in Figs. 3E and 3F, which show the frequency dependence of $|\hat{H}|^2$ over the range of $0 < \omega/2\pi < 1\cdot\text{h}^{-1}$. For fixed k_y, major gaps exist within which $\hat{C}_T$ is not much affected by environmental variability (e.g., between 9 and 24 h). For fixed k_x, however, copies of a single broad peak extend from low frequencies to about $0.17\cdot\text{day}^{-1}$, and $\hat{C}_T$ is much more susceptible to along-basin fluctuations than to cross-basin ones at low frequencies. The expected 41-min periodicity exists but cannot be seen in these plots due to their limited frequency range. However, a probably unexpected 8-h periodicity is seen in $\hat{H}$ for fixed k_y and is related to the rowwise sampling plan in which adjacent rows require 8 h to complete. This example thus confirms most of our expectations, although in a more complex fashion than anticipated. It does, however, reveal an unsuspected large difference in the ability of the sampling array to damp cross- and along-basin fluctuations that originate in the rowwise choice of the sampling plan. An alternative sampling strategy for this example is explored in Helbig and Pepin (1998).

To extend this example, we now consider the variance in estimates of the apparent mortality Z_{num} that would derive from a 5-day experiment using this sampling plan. The only changes we need to make to our discussion relate to the effects of the frequency-dependent term $f_1(\omega) = 1 - \cos(\omega\Delta T)$ in eq. 43 and the fact that relevant time scale extends to the longer limit ΔT. The component parts of the integral in eq. 43 are plotted in in Fig. 4. Figure 4A displays contours of an assumed spectrum (dash-dotted) and $|\hat{H}|^2$ (solid), both integrated over k_y to reduce the dimensionality. The regions where the two curves overlap are those that contribute to the error in the estimates (Fig. 4B). This overlap must be multiplied by the phase function f_1 (Fig. 4C) which selects a subset of frequencies that affect $\text{var}(\hat{Z}_{\text{num}})$. The overall effect is that only a relatively few, well-defined wave number – frequency zones contribute significantly to the environmentally induced variability. Figure 4B also represents the expected environmental variance in $\hat{C}_T$, which is seen to be greater than that in $\hat{Z}_{\text{num}}$.

This simple example has illustrated some of the power of the present formalism in evaluating sampling strategies. However, the true utility of our approach is for examining schemes that are nonuniform in space and (or) time. Of course, this formalism is of value only if it can be simply

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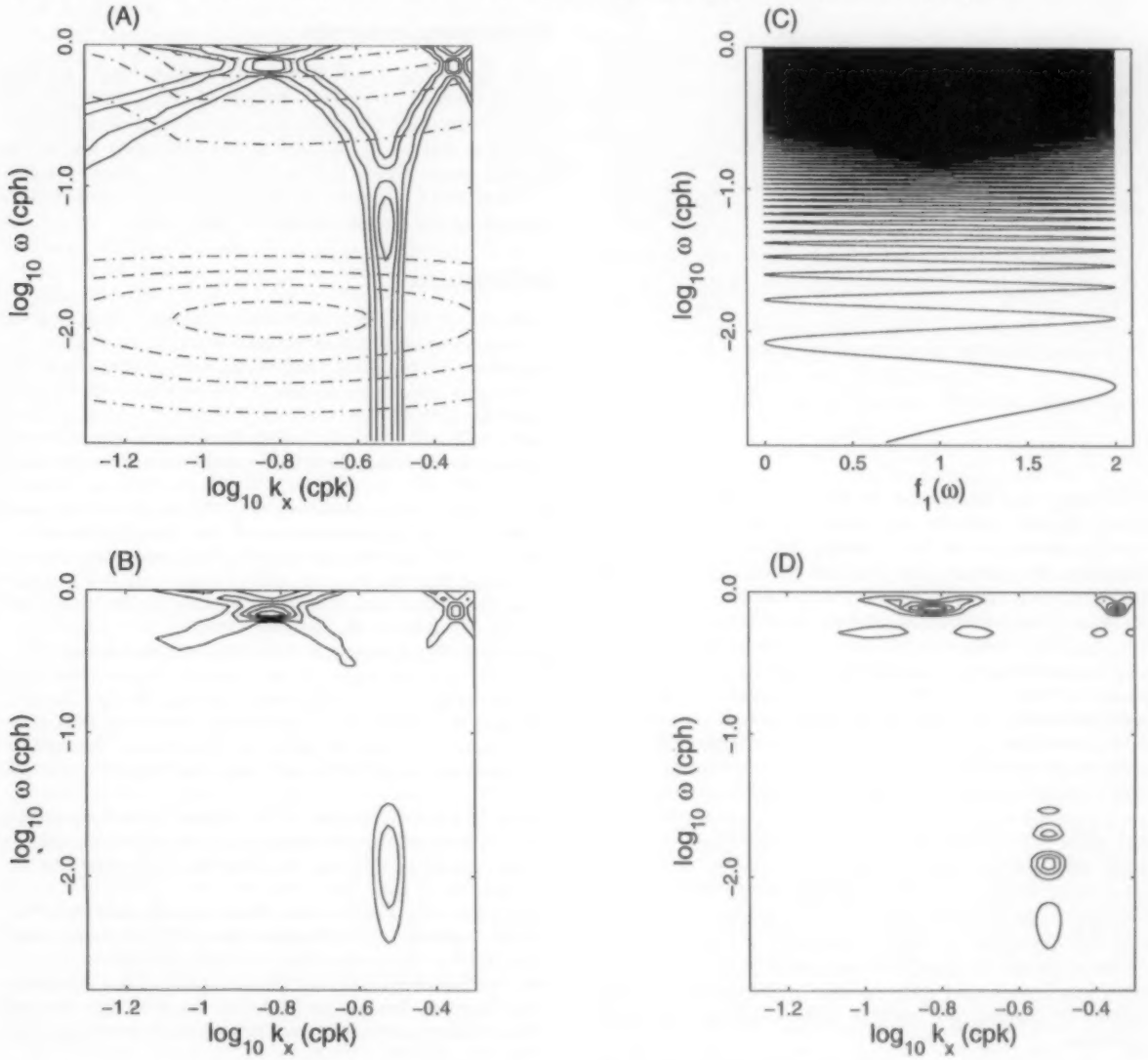
Fig. 4. Anatomy of var($\hat{Z}_{num}$). (A) Contours of $k_x\omega\int S_u(k_x,k_y,\omega)\,dk_y$ (dash-dotted) and $\int\left|\hat{H}(k_x,k_y,\omega)\right|^2 dk_y$ (solid); (B) contours of $k_x\omega\int S_u(k_x,k_y,\omega)\left|\hat{H}(k_x,k_y,\omega)\right|^2 dk_y$; (C) phase function $f_1 = (1 - \cos(\omega\Delta T))$ versus $\log(\omega)$; (D) contours of $k_x\omega\int f_1(\omega)S_u(k_x,k_y,\omega)\left|\hat{H}(k_x,k_y,\omega)\right|^2 dk_y$. The factor $k_x\omega$ corrects for the distortion induced in a log–log plot and ensures that each factor contributes to its respective integral In accord with the area subtended by its graph. cpk, cycles·km^{-1}; cph, cycles·h^{-1}.



applied in practice, and we demonstrate in Helbig and Pepin (1998) that it can. On the other hand, the task of designing an optimum sampling strategy is a complicated one that can be approached in several manners. It lies outside the scope of this paper, but it is a topic we are actively studying.

We return to the remark made earlier in this section regarding the misapplication of "traditional" sampling theory. If the purpose of a survey is to estimate an average plankton concentration, some aliasing can be tolerated. However, since the avoidance of aliasing is the most frequently voiced objective of survey design, it is instructive to further explore its significance within the context of population estimation. To do so, we need only consider the one-dimensional example of a time series $h(t) = 2 + \cos(2\pi ft)$ shown in Fig. 5 for $f = 1\cdot\text{day}^{-1}$. The top panel illustrates a typical example of aliasing in which the signal is sampled at an interval of 0.8 day and is indistinguishable from a lower frequency signal of the same amplitude but with frequency $f_a = 0.25\cdot\text{day}^{-1}$. In signal processing language, the signal frequency f is greater than the Nyquist frequency $f_N = 1/(2 \times 0.8) =$

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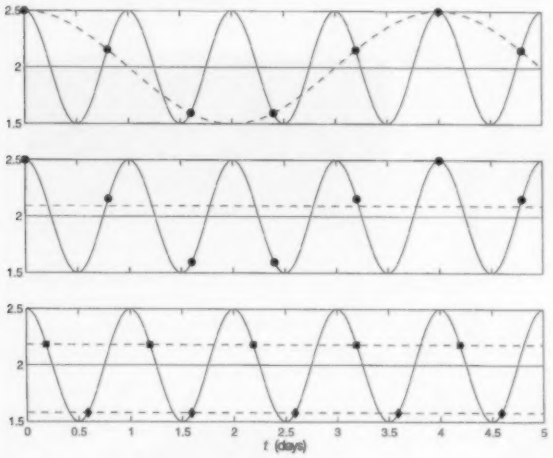
Fig. 5. Top panel: signal $h(t) = 2 + \cos(2\pi ft)$, $f = 1 \cdot \text{day}^{-1}$ (solid), sampled at an interval of 0.8 day (circles) and thus indistinguishable from the signal $h_a(t) = 2 + \cos(2\pi f_a t)$, $f_a = 0.25 \cdot \text{day}^{-1}$; middle panel: mean of a set of points sampled at at interval of 0.8 day; lower panel: means of two sets of points sampled at at interval of 1 day.



$0.625 \cdot \text{day}^{-1}$ and thus cannot be resolved. The middle panel shows that the mean of the sampled points lies relatively close to the true mean. Even though the signal is aliased by sampling, the sample mean remains useful: aliased or not, each measurement remains valid for computing a variety of statistics including the mean and variance. From an information theoretic viewpoint, two samples per cycle are necessary to unambiguously determine a signal's amplitude and phase and thus to unambiguously distinguish it from other members of the spectrum. By contrast, it is necessary only to draw samples at different phases of the signal in order to achieve an estimate of the mean and other statistics. The bottom panel shows two cases of sampling at the same frequency as the signal. In each case, the signal is sampled at one point only in its cycle, and the two estimates of the mean consequently differ greatly. This explains why $\hat{C}_T$ is sensitive to variance at frequencies and wave numbers corresponding to the sampling intervals. To conclude, we emphasize that avoidance of aliasing probably is a good rule of thumb in the design of experiments whose purpose is to estimate populations, but only because it encourages a large number of stations, not because aliasing corrupts the measurements.

For the present, we conclude by noting that to estimate the bias and variance of individual population surveys or of the apparent mortality Z_{num}, one must specify (*i*) the sampling plan including station times and locations as well as the basin size and duration of the survey, (*ii*) the spectrum for plankton fluctuations $S_c(k_x,k_y,\omega)$, (*iii*) an estimate of the experimental measurement error σ_ε, and (*iv*) a guess at the effective mortality Z_e. To estimate the bias and variance of the advective plankton flux out of a region, one must specify (*i*) the sampling plan, (*ii*) the spectra for plankton and along-channel velocity $S_c(k_x,\omega)$ and $S_v(k_x,\omega)$, as well as an estimate for the cross-spectrum $S_{vc}(k_x,\omega)$, (*iii*) estimates of the mean plankton and along-channel velocity along the open boundary $c_o(x,L_y)$ and $v_o(x,L_y)$, (*iv*) estimates of the experimental errors σ_ε and σ_v, and (*v*) a guess at the effective mortality Z_e. Most of the elements can be estimated from existing data for an area or inferred based on general knowledge of scale-dependent variability of marine ecosystems.

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References

Anderson, J.T. 1984. Early life history of redfish (*Sebastes* spp.) on Flemish Cap. Can. J. Fish. Aquat. Sci. **41**: 1106–1116.

Arai, N.M., and Hay, D.E. 1982. Predation by medusae on Pacific herring (*Clupea harengus pallasi*) larvae. Can. J. Fish. Aquat. Sci. **39**: 1537–1540.

Bailey, K.M., and Houde, E.D. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. **25**: 1–83.

Bennett, A.F., and Denman, K.L. 1985. Phytoplankton patchiness: inferences from particle statistics. J. Mar. Res. **43**: 307–335.

Blackman, R.B., and Tukey, J.W. 1959. The measurement of power spectrum from the point of view of communications engineering. Dover, New York. (Originally published in Bell Syst. Tech. J. **37**: 185–282 and **37**: 485–569, 1958.)

Castro, L.R., and Cowen, R.K. 1991. Environmental factors affecting the early life stages of bay anchovy, *Anchoa mitchilli*, in Great South Bay, New York. Mar. Ecol. Prog. Ser. **76**: 235–247.

Chavance, P., Flores-Coto, C., and Sanchez-Iturbe, A. 1984. Early life history and adult biomass of sea bream in the Terminos Lagoon, southern Gulf of Mexico. Trans. Am. Fish. Soc. **113**: 166–177.

Cowan, J.H., and Houde, E.D. 1993. Relative predation potentials of scyphomedusae, ctenophores and planktivorous fish on ichthyoplankton in Chesapeake Bay. Mar. Ecol. Prog. Ser. **95**: 55–65.

Cyr, H., Downing, J.A., Lalonde, S., Baines, S.B., and Pace, M.L. 1992. Sampling larval fish populations: choice of sampler number and size. Trans. Am. Fish. Soc. **121**: 356–368.

Daan, N., Rijnsdorp, A.D., and van Overbeeke, G.R. 1985. Predation by North Sea herring *Clupea harengus* on eggs of plaice *Pleuronectes platessa* and cod *Gadus morhua*. Trans. Am. Fish. Soc. **114**: 499–506.

Davis, T.L.O., Lyne, V., and Jenkins, G.P. 1991. Advection, dispersion and mortality of a patch of southern bluefin tuna larvae *Thunnus maccoyii* in the East Indian Ocean. Mar. Ecol. Prog. Ser. **73**: 33–45.

deLafontaine, Y., and Leggett, W.C. 1988. Predation by jellyfish on larval fish: an experimental evaluation employing in situ enclosures. Can. J. Fish. Aquat. Sci. **45**: 1173–1190.

Fancett, M.S., and Jenkins, G.P. 1988. Predatory impact of scyphomedusae on ichthyoplankton and other zooplankton in Port Phillip Bay. J. Exp. Mar. Biol. Ecol. **116**: 63–77.

Fortier, L., and Leggett, W.C. 1985. A drift study of larval fish survival. Mar. Ecol. Prog. Ser. **25**: 245–257.

© 1998 NRC Canada

Heath, M.R. 1992. Field investigations of the early life stages of marine fish. Adv. Mar. Biol. **28**: 2–174.

Heath, M.R., and MacLachlan, P. 1987. Dispersion and mortality of yolk-sac herring (*Clupea harengus*) larvae from a spawning ground to the west of the Outer Herbides. J. Plankton Res. **9**: 613–630.

Helbig, J.A., and Pepin, P. 1998. Partitioning the influence of physical processes on the estimation of ichthyoplankton mortality rates. II. Application to simulated and field data. Can. J. Fish. Aquat. Sci. **55**. This issue.

Henderson, P.A., Whitehorse, J.W., and Cartwright, G.H. 1984. The growth and mortality of larval herring, *Clupea harengus*, in the River Blackwater Estuary. J. Fish Biol. **24**: 613–622.

Hewitt, R.P., Theilacker, G.H., and Lo, N.C.H. 1985. Causes of mortality in young jack mackerel. Mar. Ecol. Prog. Ser. **26**: 1–10.

Hinckley, S., Bailey, K.M., Picquelle, S., Yoklavich, M., and Stabeno, P. 1993. Age-specific mortality and transport of larval walleye pollock *Theragra chalcogramma* in the western Gulf of Alaska. Mar. Ecol. Prog. Ser. **98**: 17–29.

Houde, E.D., Gamble, J.C., Dorsey, S.E., and Cowan, J.H. 1994. Drifting mesocosms: the influence of gelatinous zooplankton on mortality of bay anchovy, *Anchoa mitchilli*, eggs and yolk-sac larvae. ICES J. Mar. Sci. **51**: 383–394.

Isserlis, L.1918. On a formula for the product-moment coefficient in any number of variables. Biometrika, **12**: 134–139.

Kierstead, H., and Slobodkin, B. 1953. The size of water masses containing plankton blooms. J. Mar. Res. **12**: 141–147.

Kim, S., and Bang, B. 1990. Oceanic dispersion of larval fish and its implications for mortality estimates: case study of walleye pollock larvae in Shelikof Strait, Alaska. Fish. Bull. U.S. **88**: 303–311.

Kiørboe, T., Munk, P., Richardson, K., Christensen, V., and Paulsen, H. 1988. Plankton dynamics and larval herring growth, drift and survival in a frontal area. Mar. Ecol. Prog. Ser. **44**: 205–219.

Leak, J.C., and Houde, E.D. 1987. Cohort growth and survival of bay anchovy *Anchoa mitchilli* larvae in Biscayne Bay, Florida. Mar. Ecol. Prog. Ser. **37**: 109–122.

Legendre, L., and Demers, S. 1984. Towards dynamic biological oceanography and limnology. Can. J. Fish. Aquat. Sci. **41**: 2–19.

Leggett, W.C., and Deblois, E. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Neth. J. Sea Res. **32**: 119–134.

Lough, R.G., Bolz, G.R., Grosslein, M.D., and Rosenberg, A.S. 1981. Abundance and survival of sea herring (*Clupea harengus*) larvae in relation to environmental factors, spawning stock size, and recruitment for the Georges Bank area, 1968–1977. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **178**: 220–222.

McGurk, M.D. 1989. Advection, diffusion and mortality of Pacific herring larvae, *Clupea harengus pallasi*, in Bamfield Inlet, British Columbia. Mar. Ecol. Prog. Ser. **51**: 1–18.

Möller, H. 1980. Scyphomedusae as predators and food competitors of larval fish. Meeresforschung, **28**: 90–100.

Murdoch, R.C., and Quigley, B. 1994. Patch study of mortality, growth and feeding of the southern gadoid *Macruronus novaezelandiae*. Mar. Biol. **121**: 23–33.

Myers, R.A., and Cadigan, N.G. 1993. Density-dependent juvenile mortality in marine demersal fish. Can. J. Fish. Aquat. Sci. **50**: 1576–1590.

Oppenheim, A.V., and Schafer, R.W. 1975. Digital signal processing. Prentice-Hall Inc., Englewood Cliffs, N.J.

Peebles, E.B., and Tolley, S.G. 1988. Distribution, growth and mortality of larval spotted seatrout, *Cynoscion nebulosus*: a comparison between two adjacent estuarine areas of southwest Florida. Bull. Mar. Sci. **42**: 397–410.

Pepin, P. 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Can. J. Fish. Aquat. Sci. **48**: 503–518.

Pepin, P., and Helbig, J.A. 1997. The drift and distribution of cod eggs and larvae on the northeast Newfoundland Shelf. Can. J. Fish. Aquat. Sci. **54**: 670–685.

Pepin, P., and Shears, T.H. 1997. Variability and capture efficiency of bongo and Tucker trawl samplers in the collection of ichthyoplankton and other macrozooplankton. Can. J. Fish. Aquat. Sci. **54**: 765–773.

Pepin, P., Helbig, J.A., Laprise, R., Colbourne, E., and Shears, T.H. 1995. Variations in the contribution of transport to changes in planktonic animal abundance: a study of the flux of fish larvae in Conception Bay, Newfoundland. Can. J. Fish. Aquat. Sci. **52**: 1475–1486.

Peterson, I., and Wroblewski, J.S. 1984. Mortality rates of pelagic fishes in the pelagic ecosystem. Can. J. Fish. Aquat. Sci. **41**: 1117–1120.

Power, J.H. 1996. Simulations of the effect of advective–diffusive processes on observations of plankton abundance and population rates. J. Plankton Res. **18**: 1881–1896.

Priestly, M.B. 1981. Spectral analysis. Vol. 1. Academic Press, London, U.K.

Purcell, J.E. 1984. Predation on fish larvae by *Physalia physalis*, the Portuguese man of war. Mar. Ecol. Prog. Ser. **19**: 189–191.

Purcell, J.E. 1989. Predation on fish larvae and eggs by the hydromedusa *Aequorea victoria* at a herring spawning ground in British Columbia. Can. J. Fish. Aquat. Sci. **46**: 1415–1427.

Purcell, J.E., Nemazie, D.A., Dorsey, S.E., Houde, E.D., and Gamble, J.C. 1994. Predation mortality of bay anchovy *Anchoa mitchilli* eggs and larvae due to scyphomedusae and ctenophores in Chesapeake Bay. Mar. Ecol. Prog. Ser. **114**: 47–58.

Reed, R.K., Incze, L.S., and Schumacker, J.D. 1989. Estimation of the effects of flow on dispersion of larval pollock, *Theragra chalcogramma*, in Shelikof Strait, Alaska. *In* Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. *Edited by* R.J. Beamish and G.A. MacFarlane. Can. Spec. Publ. Fish. Aquat. Sci. No. 108. pp. 239–246.

Santander, H., Alheit, J., MacCall, A.D., and Alamo, A. 1983. Egg mortality of the Peruvian anchovy (*Engraulis ringens*) caused by cannibalism and predation by sardines (*Sardinops sagax*). *In* Proceedings of the Expert Consultation to Examine Changes in Abundance and Species Composition of Neritic Fish Resources. *Edited by* G.D. Sharp and J. Csirke. FAO Fish. Rep. No. 291. pp. 1011–1025.

Taggart, C.T., and Frank, K.T. 1990. Perspectives on larval fish ecology and recruitment processes: probing the scales of relationships. *In* Large marine ecosystems: patterns, processes, and yields. *Edited by* K. Sherman, L.M. Alexander, and B.D. Gold. American Association for the Advancement of Science, Washington, D.C. pp. 151–164.

Taggart, C.T., and Leggett, W.C. 1987. Short-term mortality in post emergent capelin *Mallotus villosus*. I. Analysis of multiple in situ estimates. Mar. Ecol. Prog. Ser. **41**: 205–217.

Talbot, J.W. 1977. The dispersal of plaice eggs and larvae in the Southern Bight of the North Sea. J. Cons. Cons. Int. Explor. Mer, **37**: 221–248.

Theilacker, G., Lo, N.C.H., and Townsend, A.W. 1993. An immunological approach to quantifying predation by euphausiids on the early stages of anchovy. Mar. Ecol. Prog. Ser. **92**: 35–50.

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van der Veer, H., and Bergman, M.J.N. 1987. Predation by crustaceans on a newly settled 0-group plaice *Pleuronectes platessa* population in the western Wadden Sea. Mar. Ecol. Prog. Ser. **35**: 203–215.

Ware, D.M., and Lambert, T.C. 1985. Early life history of Atlantic mackerel (*Scomber scombrus*) in the southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **42**: 577–592.

Appendix. Expressions for mortality estimators

The machinery necessary to deal with nonlinear functions of random variables as well as to convert ensemble averages of products of fluctuating variables into spectral form is presented here. In addition, a variety of expressions too cumbersome for the main text are listed.

We reemphasize that the velocity and plankton concentration fields are decomposed into mean and fluctuating parts. The means are deterministic whereas the fluctuations are taken to be stationary random processes. In a strict sense, c' and $\mathbf{u}'$ cannot be stationary in their spatial coordinates because the systems under consideration are limited in horizontal extent. This is an assumption that is frequently violated, and it has a subtle, but significant implication. In an unbounded system, $\langle c'(t) \rangle$ would identically vanish over time as would $\bar{c}'$. The restriction to a finite system destroys this constraint and means that the true value of the number of plankton in the basin varies from ensemble to ensemble (eq. 10). For finite systems, one generally assumes that the time and length scales of interest are "short" relative to the system dimensions; we henceforth assume that the spectra are band limited in space and are not energetic at length scales exceeding the basin width.

Probably the simplest way to express the fluctuations in terms of their respective auto- and cross-spectra is through the Wiener–Khinchine theorem (eq. 8). The utility of this form is demonstrated by deriving expression 29 for $\mathrm{var}(\hat{C}_T)$. We have

$$\begin{aligned}
\mathrm{var}(\hat{C}_T) &= \frac{m^2(T)}{J^2} \sum_j \sum_{j'} \mathscr{E}(c'_j c'_{j'}) \\
&= \frac{m^2(T)}{J^2} \sum_j \sum_{j'} R_c(\boldsymbol{\xi}_{j'} - \boldsymbol{\xi}_j, \tau_{j'} - \tau_j) \\
&= m^2(T) \int \mathbf{dk} \mathrm{d}\omega \, S_c(\mathbf{k}, \omega) \frac{1}{J^2} \sum_j \sum_{j'} \mathrm{e}^{i\mathbf{k}\cdot\boldsymbol{\xi}_j} \mathrm{e}^{-i\mathbf{k}\cdot\boldsymbol{\xi}_{j'}} \mathrm{e}^{-i\omega\tau_j} \mathrm{e}^{i\omega\tau_{j'}} \\
&= m^2(T) \int S_c(\mathbf{k}, \omega) \left| \hat{H}(\mathbf{k}, \omega) \right|^2 \mathbf{dk} \mathrm{d}\omega
\end{aligned}$$

Also derived in this manner are

$$\begin{aligned}
\text{(A1)} \quad \mathrm{var}(\hat{\Psi} - \Psi) \approx & \int S_v(\mathbf{k}, \omega) \left| \hat{F}_C(k_x, \omega) \hat{M}(\omega) - F_C(k_x) M(\omega) \right|^2 \mathbf{dk} \mathrm{d}\omega \\
& + \int S_c(\mathbf{k}, \omega) \left| \hat{F}_V(k_x, \omega) \hat{M}(\omega) - F_V(k_x) M(\omega) \right|^2 \mathbf{dk} \mathrm{d}\omega \\
& + 2 \int S_{vc}(\mathbf{k}, \omega) \left[\hat{F}_C^*(k_x, \omega) \hat{F}_V(k_x, \omega) \left| \hat{M}(\omega) \right|^2 - F_C^*(k_x) F_V(k_x) \left| M(\omega) \right|^2 \right] \\
& + \left(\int \mathbf{dk} \mathbf{dk}' \mathrm{d}\omega \mathrm{d}\omega' [S_c(\mathbf{k}, \omega) S_v(\mathbf{k}', \omega') + S_{vc}(\mathbf{k}, \omega) S_{vc}(\mathbf{k}', \omega')] \right. \\
& \left. \left| \hat{Q}(k_x - k'_x, \omega - \omega') \hat{M}(\omega - \omega') - Q(k_x - k'_x, \omega - \omega') M(\omega - \omega') \right|^2 \right) \\
& + \left(\sum_m \left(\frac{\Delta l_m}{L_x} \right)^2 m^2(\delta t_m) v_{cm}^2 \right) + \left(\sum_n \left(\frac{\Delta t_n}{\Delta T} \right)^2 m^2(T_{fn}) \right) \sigma_c^2 + \left(\sum_m \left(\frac{\Delta l_m}{L_x} \right) m(\delta t_m) c_{cm} \right)^2 \hat{M}^2(0) \sigma_v^2
\end{aligned}$$

and

$$\text{(A2)} \quad \mathrm{var}(\hat{C} - \bar{C}) = \left(\frac{1}{4} \int S_c(\mathbf{k}, \omega) [1 + 2m(\Delta T) \cos(\omega \Delta T) + m^2(\Delta T)] \left| \hat{H}(\mathbf{k}, \omega) - H(\mathbf{k}) M(\omega) \right|^2 \mathbf{dk} \mathrm{d}\omega \right) + \frac{1 + m^2(\Delta T)}{4J'} \sigma_c^2$$

where

$$\text{(A3)} \quad F_C(k_x) = \frac{1}{L_x} \int_{L_x} c_o(x, L_y) \mathrm{e}^{ik_x x} \, \mathrm{d}x$$

$$\text{(A4)} \quad F_V(k_x) = \frac{1}{L_x} \int_{L_x} v_o(x, L_y) \mathrm{e}^{ik_x x} \, \mathrm{d}x$$

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$$\text{(A5)}\quad Q(k_x) = \frac{1}{L_x}\int_{L_x} e^{ik_x x}\,dx = e^{ik_x L_x/2}\,\frac{\sin(k_x L_x/2)}{k_x L_x/2}$$

$$\text{(A6)}\quad M(\omega) = \frac{1}{\Delta T}\int_{L_t} m(t)e^{-i\omega t}\,dt = \frac{1}{\Delta T}\,\frac{Z_e - i\omega}{Z_e^2 + \omega^2}\,(1 - e^{-Z_e \Delta T}e^{i\omega\Delta T})$$

are the continuous analogues of eqs. 36–39. Equations like A1 and A2 actually embody the expressions for both the realistic and true values, as can be seen by comparing eqs. 28 and 29 with eq. 32. Consequently, we use this form for economy in the remainder of the Appendix.

Mortality estimates

Computation of the statistics of the mortality estimates is complicated by the fact that the expressions are nonlinear in the stochastic variables, thus prohibiting straightforward ensemble averages. However, provided that the fluctuations are small compared with the mean, these expressions can be expanded in series of deterministic and purely random terms. Specifically, for $\delta = C'/C_o$, we utilize expressions of the form

$$\text{(A7)}\quad \frac{1}{\hat{C}} = \frac{1}{\hat{C}_o}\left[1 - \frac{\hat{C}_1 + \hat{C}_2}{\hat{C}_o} + \left(\frac{\hat{C}_1 + \hat{C}_2}{\hat{C}_o}\right)^2 + O(\delta^3)\right]$$

and

$$\text{(A8)}\quad \ln\hat{C}_T = \ln\hat{C}_{oT} + \left[\frac{\hat{C}'_T}{\hat{C}_{oT}} - \frac{1}{2}\left(\frac{\hat{C}'_T}{\hat{C}_{oT}}\right)^2 + O(\delta^3)\right].$$

Using these expansions and discarding all terms involving correlations higher than second order, we find eqs. 43 and 44 as well as

$$\text{(A9)}\quad \mathcal{E}(Z_{\text{num}}) = \mathcal{E}(\hat{Z}_{\text{num}}) = Z_e$$

$$\text{(A10)}\quad \text{bias}(\hat{Z}_{\text{flux}}) =$$

$$-\frac{L_x}{A\hat{C}_o}\left\{(\hat{\Psi}_o - \beta\Psi_o) + [\hat{Q}(0,0)\hat{M}(0) - \beta M(0)]R_{vc}(\mathbf{0},0)\right.$$

$$+\frac{1}{2\hat{C}_o}\int e^{ik_y L_y} S_c(\mathbf{k},\omega)[(1 + m(\Delta T)e^{i\omega\Delta T})\hat{F}_V(k_x,\omega)\hat{M}(\omega)\hat{H}(\mathbf{k},\omega) - \beta F_V(k_x)M(\omega)H(\mathbf{k})]\,d\mathbf{k}d\omega$$

$$\left.+\frac{1}{2\hat{C}_o}\int e^{ik_y L_y} S_{vc}(\mathbf{k},\omega)[(1 + m(\Delta T)e^{i\omega\Delta T})\hat{F}_C(k_x,\omega)\hat{M}(\omega)\hat{H}(\mathbf{k},\omega) - \beta F_C(k_x)M(\omega)H(\mathbf{k})]\,d\mathbf{k}d\omega\right\}$$

$$\text{(A11)}\quad \text{var}(\hat{Z}_{\text{num}} - Z_{\text{num}}) = +\frac{2}{\Delta T^2 \hat{C}_{o1}^2}\,\frac{\sigma_c^2}{J'}\,\frac{2}{\Delta T^2 C_{o1}^2}\int S_c(\mathbf{k},\omega)[1 - \cos(\omega\Delta T)]\left|\hat{H}(\mathbf{k},\omega) - \beta H(\mathbf{k})\right|^2 d\mathbf{k}d\omega$$

and

$$\text{(A12)}\quad \text{var}(\hat{Z}_{\text{flux}} - Z_{\text{flux}}) = \left(\frac{L_x}{A\hat{C}_o}\right)^2\left\{\text{var}(\hat{\Psi} - \beta\Psi) + \frac{1}{\hat{C}_o^2}(\hat{\Psi}_o^2\,\text{var}(\hat{C}') - \beta^2\Psi_o^2\,\text{var}(\overline{C}'))\right.$$

$$\left.+\frac{2}{\hat{C}_o}(\hat{\Psi}_o\mathcal{E}[\hat{C}'(\hat{\Psi}_1 + \hat{\Psi}_2)] - \beta\Psi_o\mathcal{E}[\overline{C}'(\Psi_1 + \Psi_2)]) + \hat{\Psi}_o^2\,\frac{1 + m^2(\Delta T)}{J'}\,\sigma_c^2\right\}$$

with

$$\text{(A13)}\quad \mathcal{E}(\Psi_1\overline{C}' + \Psi_2\overline{C}') = \int e^{-i(k_x L_x - k_y L_y)/2} H^*(\mathbf{k})\left|M(\omega)\right|^2[S_c(\mathbf{k},\omega)F_V(k_x) + S_{vc}(\mathbf{k},\omega)F_C(k_x)]\,d\mathbf{k}d\omega$$

$$\text{(A14)}\quad \mathcal{E}(\hat{\Psi}_1\hat{C}' + \hat{\Psi}_2\hat{C}') = \int e^{-i(k_x L_x - k_y L_y)/2}\hat{H}^*(\mathbf{k})\left|\hat{M}(\omega)\right|^2[S_c(\mathbf{k},\omega)\hat{F}_V(k_x) + S_{vc}(\mathbf{k},\omega)\hat{F}_C(k_x)]\,d\mathbf{k}d\omega$$

where $\beta = \hat{C}_o/\overline{C}_o$. We note that the third term in eq. A10 is $\mathcal{E}(\hat{\Psi}_3 - \Psi_3)$.

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Partitioning the influence of physical processes on the estimation of ichthyoplankton mortality rates. II. Application to simulated and field data

J.A. Helbig and P. Pepin

Abstract: Helbig and Pepin (1998. Can. J. Fish. Aquat. Sci. **55**: 2189–2205) developed a theory partitioning the bias and variance of a number of population and mortality measures in terms of the space and time scales of natural variability of the underlying current and plankton fields. This study verifies that theory through a series of numerical simulations and by comparing predicted variances in estimated fluxes with those observed in an earlier field study. In the simulations, errors in estimated mortality rates were clearly dominated by errors in estimated fluxes due to transport, and estimates of variance and bias were all in good agreement with theoretical predictions. For the field data, the theory predicted a distribution of values slightly lower than that observed, but of the correct order. That the model performed well for both simulated and field data suggests that it can be applied in practice with reasonable confidence. We also briefly explore the role of the model in survey design and analysis for ichthyoplankton studies in which transport might be important.

Résumé : Helbig et Pepin (1998. Can. J. Fish. Aquat. Sci **55** : 2189–2205) ont élaboré une théorie pour la répartition du biais et de la variance de diverses mesures de population et de mortalité faisant appel aux échelles d'espace et de temps de la variabilité naturelle du courant sous-jacent et des zones de plancton. L'étude vérifie le bien-fondé de cette théorie de par une série de simulations numériques et une comparaison des variances prévues des flux estimés avec les valeurs correspondantes obtenues au cours d'une étude antérieure sur le terrain. Pour les simulations, les erreurs des taux de mortalité estimés étaient clairement dominées par les erreurs des flux estimés causées par le transport et les estimations de la variance et du biais correspondaient toutes aux prévisions théoriques. En ce qui a trait aux données de terrain, la théorie prévoyait une distribution des valeurs légèrement inférieure à celle observée, mais l'ordre était le bon. Le fait que le modèle fonctionne bien avec les données simulées et réelles porte à croire qu'il puisse être appliqué avec un degré de confiance raisonnable. Nous traitons aussi brièvement de l'application du modèle à la conception et à l'analyse de relevés dans le cadre d'études de l'ichtyoplancton où le transport pourrait jouer un rôle important.

[Traduit par la Rédaction]

Introduction

To assign a causal mechanism to perceived (i.e., estimated) changes in abundance of planktonic organisms, it is essential that we determine the losses attributable to transport. Success requires knowledge of the accuracy (bias) and precision (variance) of a field study in order to be confident of inferences. This is of particular importance in dealing with prerecruitment mortality, as consensus concerning the major factors that influence survival during the pelagic egg and larval stages is lacking (Leggett and Deblois 1994).

Helbig and Pepin (1998) (hereafter referred to as HPI) presented a theoretical framework showing how error-free measures of plankton population, transport, and mortality vary with naturally occurring environmental fluctuations. Field-based estimates of these quantities vary to an even greater degree, as experiments cannot adequately sample all the variability in the plankton and velocity fields. In essence, the problem addressed by HPI is contained in the expression

$$(1) \qquad \hat{Z}_{\text{num}} = Z + \hat{Z}_{\text{flux}} + \hat{Z}_{\text{diff}}$$

relating the *apparent* mortality $\hat{Z}_{\text{num}}$ estimated from the difference in plankton concentration between surveys to the the true biological mortality Z, the loss due to transport $\hat{Z}_{\text{flux}}$, and the diffusive loss $\hat{Z}_{\text{diff}}$. HPI showed how the bias and variance of these terms were partitioned according to the space and time scales of variability of the underlying current and plankton fields. As well, HPI indicated that their theory could be used to (*i*) evaluate sampling plans to determine the length and times scales on which estimates are vulnerable to degradation by environmental variations, (*ii*) estimate the bias and variance of the estimates for a given sampling plan and some knowledge of the current and plankton fields, and (*iii*) ultimately provide ichthyoplankton ecologists a tool for the "optimum" design of field programs prior to field experiments.

The primary purpose of this paper is to verify the HPI framework through a series of numerical simulations and by comparing predicted variances of $\hat{Z}_{\text{flux}}$ with those observed in the field. In addition, a brief evaluation of alternative

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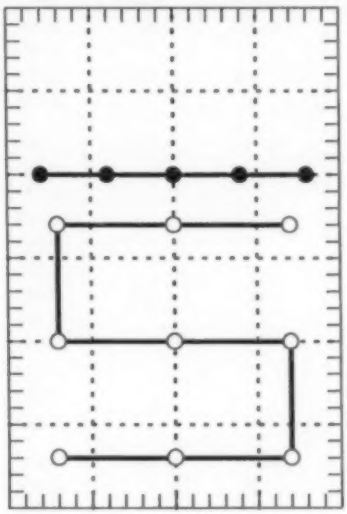
Fig. 1. Basin geometry for the numerical simulations of a 20 × 20 km study system. Tick marks represent 1-km intervals. Open circles show the location of stations used in the basin surveys, and closed circles show the location of stations sampled in the flux surveys. The solid lines represent ship tracks.



sampling plans is made to illustrate how the framework might be used for array design.

Numerical simulations

A series of mortality estimation experiments were simulated by stepping the larval balance equation

$$\frac{\partial c}{\partial t} = -Zc - \nabla \cdot (\mathbf{u}c) + K_H \nabla^2 c \quad (2)$$

through time and sampling the resultant larval and current fields appropriately. Here, $c(x,y,t)$ is the plankton concentration (number per cubic metre), $\mathbf{u} = (u,v)$ is the horizontal velocity, and K_H is the horizontal diffusivity. Numerical experiments were run for a series of basins (widths of L = 5, 10, 20, 50, 100, and 200 km) with a single boundary open to the deep ocean using a fixed grid spacing Δx = 1 km (Fig. 1). Each experiment was run 100 times with current fields randomly drawn from an appropriately defined ensemble.

We avoided specifying open boundary conditions by modelling the plankton distribution within a rectangular, closed basin of size $[L \times (L + 10)]$ km and artificially inscribing an open boundary across which the advective and diffusive fluxes were measured (Fig. 1). These simulations were intended to be as realistic as possible. A time- and space-varying current field was prescribed that transported an initially specified larval field for periods up to 15 days. Larval concentration was estimated at the beginning and end of each experiment by sampling the plankton field nonsynoptically at a fixed set of stations; the measurements were corrupted to simulate experimental error. In the period separating the full-basin surveys, the flux of larvae across the open boundary was measured nonsynoptically in similar fashion at a specified set of stations. Variability was introduced into both the full-basin and flux surveys by the natural variability in the current and larval fields, which were modelled stochastically.

In any given experiment, the velocity field was drawn from an ensemble of fields constrained only by a very limited knowledge of the true field. Because the larvae were assumed to be passive drifters, the plankton field was also stochastic although not independent of the velocity field. The current and larval fields were decomposed into mean and fluctuating parts as

$$\mathbf{u}(\mathbf{x},t) = \mathbf{0} + \mathbf{u}'(\mathbf{x},t) \quad (3)$$

$$c(\mathbf{x},t) = m(t)c_o + m(t)c'(\mathbf{x},t) \quad (4)$$

with

$$\mathcal{E}(c_o) = c_o, \quad \mathcal{E}(c') = 0, \quad \mathcal{E}(\mathbf{u}') = \mathbf{0} \quad (5)$$

where $\mathcal{E}$ denotes an ensemble average and the nondimensional, deterministic function

$$m(t) = e^{-Zt} \quad (6)$$

expresses the population decline due to mortality. (Note that true mortality appears here, not the effective mortality defined in HPI. This is one benefit of working within a closed system.) The underlying natural mortality Z was set at 0.1·day^{-1}. Our choice includes a zero mean velocity field and a spatially constant mean plankton field and thus focusses attention the variability in the velocity and plankton fields.

Currents were drawn from an ensemble defined by the wave number–frequency spectra $S_u(\mathbf{k},\omega) = S_v(\mathbf{k},\omega) = S(k,\omega)$ where

$$S(k,\omega) = \begin{cases} Ak^{-2}\omega e^{-\omega/\omega_m} & k_l \le k \le k_h,\ \omega_l \le \omega \le \omega_h \\ + Bk^{-2} & k_0 \le k \le k_h,\ \omega_0 \le \omega \le \omega_h \end{cases} \quad (7)$$

represents a horizontally isotropic process broadly peaked in frequency about a period of 5 days ($\omega_m/2\pi$ = 0.2·day^{-1}) together with an additional high-frequency, high wave number plateau. Frequencies extend over the range of $\omega_l/2\pi$ = 1/(25 days) to $\omega_h/2\pi$ = 1/(2 h). The upper wave number limit of $k_h/2\pi$ = 1/(2 km) was chosen to represent the smallest possible length scale resolvable by the model (model Nyquist wave number), while the lower limit k_l varied with basin size and was chosen to admit fluctuations no larger than the basin width:

$$2\pi/k_l = \min(L, 100)\text{km}.$$

The frequency-dependent portion of the spectrum was meant to represent directly wind-driven currents and eddies spawned by inertial instabilities that are common in coastal systems (e.g., Smith and Schwing 1991). Currents in such systems are most energetic in the "weather" band extending over a range of about 3–10 days. The wave number part of the spectrum is consistent with the relatively few measures of the spatial variability available. For example, J.A. Helbig and P. Pepin (unpublished data) indicate that currents measured in the top metre of a 20 × 50 km bay with high-frequency groundwave radar had a spectrum proportional to k^{-2} on scales extending from 2 to at least 20 km

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Table 1. List of numerical experiments run classified by basin size L, number of basin survey stations J, number of flux survey stations M, number of flux surveys N, and simulation duration ΔT and indicating the duration of basin survey T_{basin}, duration of flux survey T_{flux}, the root mean square speed σ_u, and the biases $\hat{Z}_{num}/Z$ and $\hat{Z}_{flux}/Z$ and the standard deviations of $\hat{Z}_{num}/Z$ and $\hat{Z}_{flux}/Z$.

Run	L (km)	ΔT (days)	J	M	N	T_{basin} (h)	T_{flux} (h)	σ_u ($m \cdot s^{-1}$)	Bias $\hat{Z}_{num}/Z$	SD $\hat{Z}_{num}/Z$	Bias $\hat{Z}_{flux}/Z$	SD $\hat{Z}_{flux}/Z$
1	5	5	25	5	3	14	3	0.054	0.003	0.06	1.307	1.199
2				3			2				0.874	2.354
3			9	5		5	3		0.011	0.135	1.303	1.194
4				3			2				0.858	2.373
5	10	5	25	10	3	15	6	0.075	–0.012	0.116	0.860	0.767
6					2						0.895	0.858
7					1						0.754	1.309
8				5	3		3				0.829	1.012
9					2						0.884	1.174
10					1						0.666	1.763
11			9	10	3	6	6		–0.006	0.207	0.863	0.770
12					2						0.899	0.861
13					1						0.755	1.306
14				5	3		3				0.833	1.020
15					2						0.887	1.183
16					1						0.673	1.760
17	20	5	36	10	3	24	6	0.088	0.017	0.149	0.476	0.507
18					2						0.926	1.196
19					1						1.026	1.684
20				5	3		3				0.358	0.600
21					2						0.370	0.709
22					1						0.329	1.181
23			9	10	3	8	6		0.011	0.349	0.476	0.515
24					2						0.934	1.209
25					1						0.995	1.664
26				5	3		3				0.359	0.602
27					2						0.372	0.719
28					1						0.331	1.155
29	50	15	81	20	9	64	13	0.100	0.004	0.057	0.148	0.102
30					5						0.161	0.117
31					3						0.175	0.146
32				10	9		8				0.120	0.151
33					5						0.309	0.222
34					3						0.339	0.290
35			49	20	11	43	13		0.012	0.066	0.169	0.103
36				10	11		8				0.140	0.143
37			25	20	13	26	13		0.014	0.115	0.200	0.142
38				10	13		8				0.178	0.191
39		10	49	20	6	43	13		–0.009	0.095	0.162	0.191
40					3						0.169	0.192
41					2						0.177	0.229
42				10	6		8				0.126	0.296
43					3						0.102	0.105
44					2						0.104	0.158
45			25	20	8	26	13		0.017	0.126	0.092	0.098
46				10	8		8				0.094	0.145
47		5	25	20	3	26	13		0.039	0.350	0.080	0.089
48				10	3		8				0.081	0.125
49	100	15	81	20	7	88	15	0.111	–0.008	0.085	0.073	0.069
50					4						0.077	0.087
51					3						0.076	0.088

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Table 1 (*concluded*).

Run	L (km)	ΔT (days)	J	M	N	T_{basin} (h)	T_{flux} (h)	σ_u (m·s^{-1})	Bias $\hat{Z}_{conn}/Z$	SD $\hat{Z}_{conn}/Z$	Bias $\hat{Z}_{flux}/Z$	SD $\hat{Z}_{flux}/Z$
52				10	7		10				0.062	0.081
53					4						0.157	0.166
54					3						0.155	0.172
55			64	20	9	75	15		0.006	0.089	0.076	0.062
56				10	9		10				0.071	0.086
57			36	20	11	50	15		0.003	0.100	0.079	0.054
58					6						0.082	0.071
59					4						0.071	0.087
60				10	11		10				0.079	0.083
61					6						0.079	0.103
62					4						0.080	0.136
63		10	36	20	6	50	15		0.012	0.174	0.090	0.076
64				10	6		10				0.096	0.116
65	200	15	49	20	7	99	20	0.111	0.005	0.126	0.139	0.152
66					4						0.143	0.178
67					3						0.135	0.194
68				10	7		15				0.142	0.212
69					4						0.288	0.356
70					3						0.271	0.391
71			25	20	9	65	20		0.001	0.194	0.160	0.127
72					5						0.162	0.148
73					3						0.134	0.188
74				10	9		15				0.122	0.194
75					5						0.118	0.213
76					3						0.161	0.276

($k = [k_x^2 + k_y^2]^{1/2}$ is the wave number magnitude). Similarly, Firing (1993) reported upper layer currents measured with an acoustic Doppler current profiler at midlatitudes in the North Pacific Ocean that exhibited a k^{-2} dependence over the range of 10 to >100 km. The additional small-scale plateau was chosen to mimic small-scale "noise" present in realistic systems that is characteristic of more transient wind-induced fluctuations and larger scale turbulence (at scales near and below the internal Rossby radius of about 5–10 km). We selected the cutoff wave number and frequency as $k_o/2\pi = 1/(10\text{ km})$ and $\omega_o/2\pi = 1/(18\text{ h})$, respectively.

A and B were fixed to achieve a maximum variance of $(0.10\text{ m·s}^{-1})^2$ from the major component of the spectrum and an additional variance of $(0.05\text{ m·s}^{-1})^2$ from the small-scale plateau. The maximum variance was realized for L = 100 and 200 km, while the simulated variance for the smaller basins was correspondingly lower (Table 1). The small-scale variance was constant in all runs. We emphasize that the simulated currents were modest, with typical mean speeds of 0.10 m·s^{-1} and peak values up to four time larger. The actual method of simulating currents from the spectrum is described in the Appendix.

Although the choice of $S_u = S_v$ is the most expedient to achieve the desired distribution of variance in the velocity field, it is not the most general one, as it effectively treats u and v as independent scalars (Monin and Yaglom 1975, chap. 6). This has implications for the form of the eddies that comprise the fluctuations, as the cross-spectrum S_{uv} vanishes and the spectrum of the divergence is relatively energetic. These considerations reemphasize the fact that the simulated current field was not constrained to conform to a simple choice of dynamics (cf. Bennett and Denman (1985) who study plankton patchiness for a quasi-geostrophic velocity field with vanishing horizontal divergence). An alternative approach would be to specify forms for the the divergence and kinetic energy spectra and to deduce the appropriate forms for S_u and S_v. That exercise lies beyond the objectives of the simulations here, the most important of which was to verify the forms for the variance and bias given by HPI. We note, however, that our divergence field may be overly energetic at large length scales, where the geostrophic constraint is likely to hold. Maximum model values of divergence were about 10^{-4} s^{-1}, large but not unrealistic.

The initial plankton field for each simulation was drawn from an ensemble defined by a space-independent mean c_o = 0.2 m^{-3} and a two-dimensional horizontally isotropic wave number spectrum proportional to k^{-2} with a variance of $(0.08\text{·m}^{-3})^2$. In practice, however, the initial form of the plankton field proved unimportant, as it adjusted very quickly to the imposed velocity field. Each experiment was run for 24 h without mortality prior to sampling to allow relaxation to equilibrium.

The only boundary conditions imposed were that the normal component of both velocity and larval concentration gradient vanish at all solid boundaries. The model used standard second-order, centred-in-space, centred-in-time finite differences (Roache 1976) and was a scaled down and modi-

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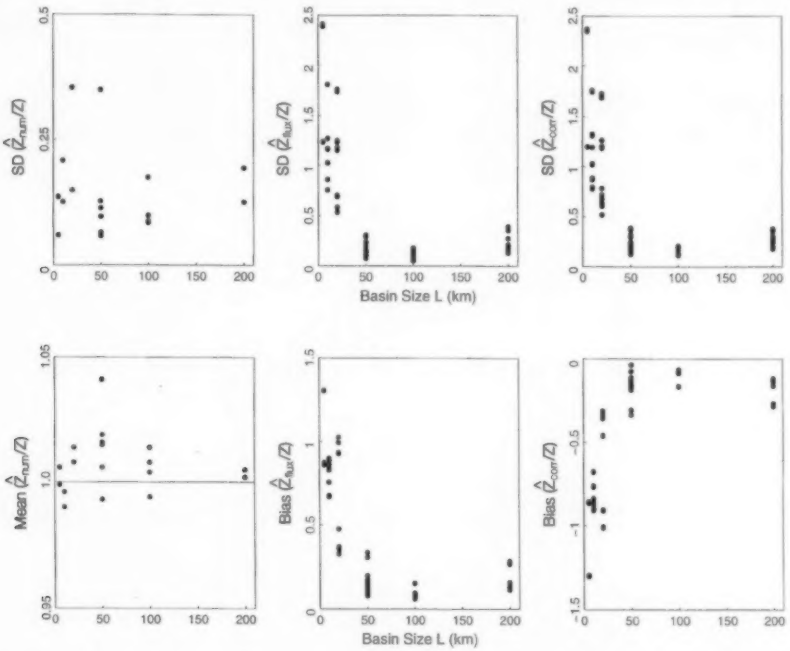
Fig. 2. Results of the numerical simulations showing means or biases and standard deviations of $\hat{Z}_{num}/Z$, $\hat{Z}_{flux}/Z$, and $\hat{Z}_{corr}/Z = (\hat{Z}_{num} - \hat{Z}_{flux} - Z_{diff})/Z$ versus basin size for the 76 sampling plans listed in Table 1. The values of Z_{diff} used to correct $\hat{Z}_{corr}$ were computed during the simulations without error. Note that the ordinate scale used for $\hat{Z}_{num}$ differs from that used for $\hat{Z}_{flux}$ and $\hat{Z}_{corr}$.



fied version of the SPEM model (Haidvogel et al. 1991). A time step of 10 min was used in all simulations, but model stability appeared to be determined by the value of te horizontal Péclet number $Pe = V\Delta x/K_H$ where V is a characteristic speed. As shown by Weaver and Sarachik (1990), stability in most centred difference schemes requires that $Pe \leq 2$. For V = 0.10 m·s^{-1}, this demands that $K_H \geq 50$ m^2·s^{-2}, near the upper end of values estimated in the field for eddy diffusivities for small systems from drifter data (e.g., deYoung and Sanderson 1995) and probably an order of magnitude larger than the small-scale eddy diffusivity for tracers (Ledwell et al. 1993). We used a value of 100 m·s^{-1} is all simulations.

The choice of geometry, in which the open boundary was embedded within the basin, and the fact that the dynamics were uniform throughout the full basin dictated that the average concentration on both sides of the open boundary must be the same. The model achieved this balance through the joint action of advection and diffusion, and the size constraint on K_H coupled with the absence of a mean flow (small advection) led to roughly equal magnitudes for both. Diffusive effects were monitored during the simulations, and some of the results presented in the next section have been corrected for them as indicated. We denote this correction as Z_{diff} (without caret) to indicate that it is measured without error.

Table 1 summarizes the 76 sampling plans that were explored in the simulations in terms of basin size, the number of basin and open boundary stations, the number of flux surveys, the overall duration of the experiment, and the times required to complete basin and flux surveys. In each case, the basin and flux stations were symmetrically arrayed (Fig. 1). Basin stations were occupied consecutively along cross-basin transects. Flux surveys were spaced symmetrically in time between the two basin surveys and were centred on noon of any given day. Between-station times were estimated assuming 30 min on station and a steaming speed of 10 knots between stations. Each realistic estimate of plankton concentration and velocity was corrupted by randomly drawing from normal distributions with zero means and respective standard deviations of 0.02·m^{-3} and 0.001 m·s^{-1}. Any corrupted plankton estimates with negative values were set equal to zero, but these were very few.

These sampling plans range from very good to rather poor. The worst are obviously bad, while some of the best are probably not logistically feasible, either in the field or later in terms of sample analysis. The "best" 50-km basin survey, for example, requires 64 h to complete. The results of the next section should be interpreted with these comments in mind.

Simulation results

Experimental results are summarized in terms of the normalized means, biases, and variances of $\hat{Z}_{num}$, $\hat{Z}_{flux}$, and the corrected mortality $\hat{Z}_{corr} = \hat{Z}_{num} - \hat{Z}_{flux} - Z_{diff}$ (Fig. 2; Table 1). Note from eq. 1 that $\hat{Z}_{corr}$ coincides with Z if the flux and diffusive corrections are accurately determined. Since the ensemble means of $\hat{Z}_{num}$, $\hat{Z}_{flux}$, and Z_{diff} are unknown analytically (they depend on the unknown correlation between

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Fig. 3. Scatterplots of predicted versus simulated variance and and bias in $\hat{Z}_{num}$ and $\hat{Z}_{flux}$. (A) var ($\hat{Z}_{num}/Z$); (B) var($\hat{Z}_{flux}/Z$). The predicted values were computed with the HPI formulation; the simulated values were calculated from the six sets of 100 numerical experiments each.

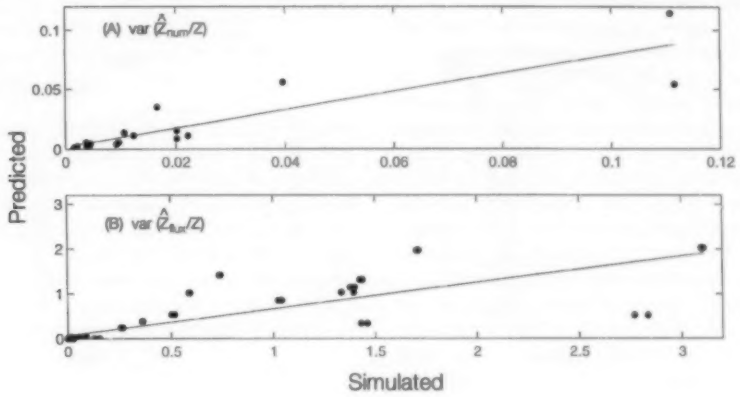
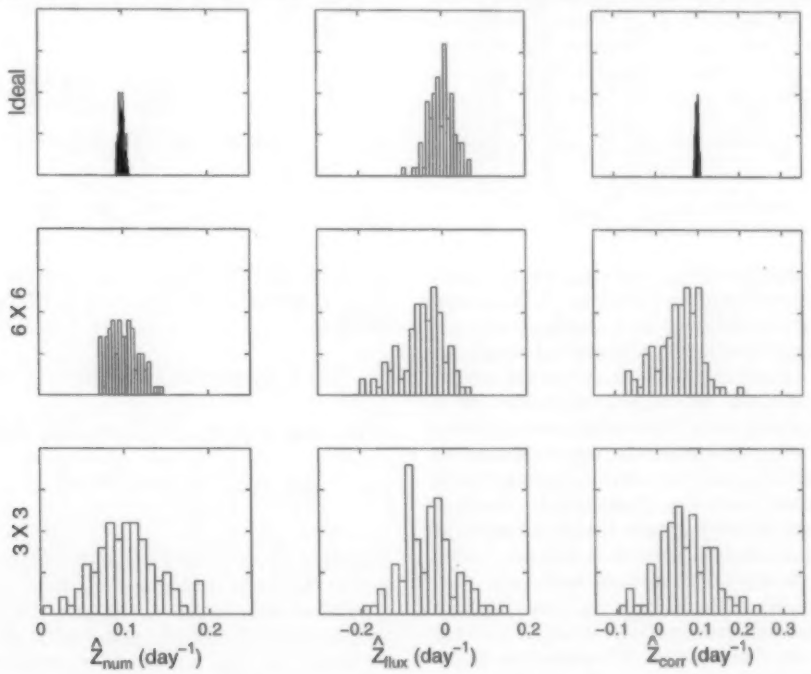


Fig. 4. Histograms of the true and estimated values of $\hat{Z}_{num}$, $\hat{Z}_{flux}$, and $\hat{Z}_{corr} = \hat{Z}_{num} - \hat{Z}_{flux} - Z_{diff}$ for numerical experiments 17 and 23 (20-km basin, 5 days). Plan 17 uses a symmetric 6 × 6 station grid coupled with three flux surveys of 10 stations each. Plan 26 uses a 3 × 3 grid coupled with three flux surveys of five stations each. The value of Z_{diff} was computed during the simulations without error.



v' and c' on the open boundary) and are needed to compute bias and variance, we computed them numerically during the simulations. Results are shown here for the case of no measurement error, i.e., for $\epsilon_c = \epsilon_v = 0$; the relative effect of measurement error is discussed later.

Errors in $\hat{Z}_{corr}$ were clearly dominated by variations in $\hat{Z}_{flux}$ (Fig. 2) which exceeded 100% for some plans for small basins. Even with the L^{-2} scaling indicated in HPI, they ranged upwards to 50% for L = 200 km. By contrast, the corresponding fractional standard deviation in $\hat{Z}_{num}$ varied from 5 to 20% for most sampling plans, although it too was especially large for small systems for some sampling plans. Interestingly, the variances in $\hat{Z}_{num}$ and $\hat{Z}_{flux}$ attained minima at L = 100 km and thereafter increased. The mean value of $\hat{Z}_{num}$ was near the true value Z and differences diminished with increasing L, while fractional biases in $\hat{Z}_{flux}$ exceeded 50% at small scales and decreased to 10–20% for larger basins. These biases were positive for all systems, meaning that the advective contribution was routinely overestimated and that Z would be underestimated if corrected for advection.

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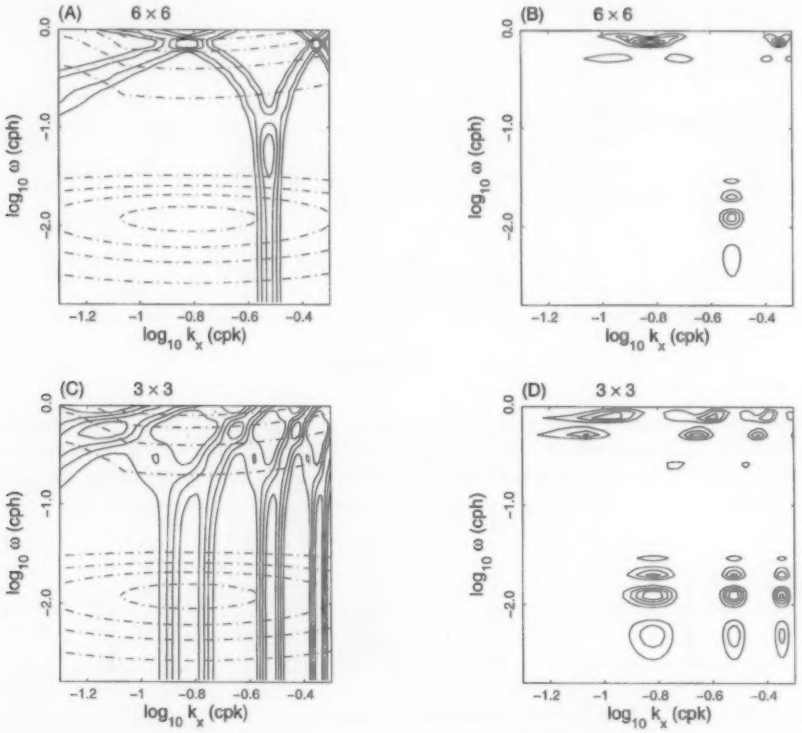
Fig. 5. Comparison of a 6 × 6 and 3 × 3 sampling plan for a 20-km basin. (A and C) Contours of $k_x\omega\int S_u(k_x, k_y, \omega)\,dk_y$ (dash-dotted) and $\int|H(k_x, k_y, \omega)|^2\,dk_y$ (solid); (B and D) contours of $k_x\omega\int S_u(k_x, k_y, \omega)(1 - \cos\omega\Delta T)|H(k_x, k_y, \omega)|^2\,dk_y$. The factor $k_x\omega$ corrects for the distortion induced in a log–log plot and ensures that each factor contributes to its respective integral in accord with the area subtended by its graph. cph, cycles·h^{-1}; cpk, cycles·km^{-1}.



Estimates of the variance in $\hat{Z}_{num}$ and $\hat{Z}_{flux}$ were in good agreement with those predicted by HPI (Fig. 3), as 80 and 65% of the variance in var($\hat{Z}_{num}$) and var($\hat{Z}_{flux}$) was explained by the leading term in the respective formula derived in HPI (eqs. 42 and 43), with S_c approximated by var(c)/var(v)S_v). In particular, the fall and subsequent rise in both var($\hat{Z}_{num}$) and var($\hat{Z}_{flux}$) with basin size were predicted.

Measurement error had little effect on the estimations of the biases of $\hat{Z}_{num}$ and $\hat{Z}_{flux}$, as values were generally within 5% of the noncorrupted estimates. Similarly, the standard deviations of $\hat{Z}_{flux}$ were only a few percent higher than those plotted. Standard deviations of $\hat{Z}_{num}$ were, in general, 5–25% larger than those in its noncorrupted counterpart.

Histograms of the 100 values of $\hat{Z}_{num}$, $\hat{Z}_{flux}$, and $\hat{Z}_{corr}$ are shown for two sampling plans for a 5-day survey of a 20-km basin (Fig. 4). The first plan utilized 36 symmetrically arrayed stations (run 17, Table 1) coupled with three flux surveys of 10 stations each whereas the second used nine basin stations aligned in three rows of three (run 26) together with three flux surveys of five stations each. The top panels in Fig. 4 illustrate the natural variability in $\hat{Z}_{num}$ and $\hat{Z}_{flux}$ whereas the middle and bottom panels demonstrate how sampling survey design induced variability overwhelms the natural variability, at least in the systems simulated. The superiority of the 36-station plan over the nine-station plan is evident.

The dependence on sampling plan is easily understood in terms of the HPI theory. For example, the variability in $\hat{Z}_{num}$, as given by the first term in HPI's eq. 42, is proportional to

$$\int S_c(\mathbf{k}, \omega)[1 - \cos(\omega\Delta T)]\left|\hat{H}(\mathbf{k}, \omega)\right|^2 d\mathbf{k}d\omega$$

where $\hat{H}(\mathbf{k}, \omega)$ is the complex-valued, deterministic function

$$(8)\qquad \hat{H}(\mathbf{k}, \omega) \approx \frac{1}{J}\sum_{j=1}^{J} e^{i(\mathbf{k}\cdot\boldsymbol{\xi}_j - \omega\tau_j)}$$

defined in terms of the location $\boldsymbol{\xi}_j$ of each of J stations relative to the basin centre and the time lag τ_j of the station occupation time relative to the central time T of the survey. Contour plots of $S_c(\mathbf{k},\omega)$ (dash-dotted) and $|\hat{H}(\mathbf{k}, \omega)|^2$ (solid) are shown in Figs. 5A and 5C for the two sampling plans whereas contour plots of the full integrand $S_c(1 - \cos\omega\Delta T)|\hat{H}|^2$ are given in Figs. 5B and 5D. These figures dramatically illustrate that the nine-station plan is vulnerable to natural environmental fluctuations over a much wider range of space and time scales than is the 36-station plan. In fact, the ratio, 6.21, of the integrals (i.e., the areas shown in Figs. 5B and 5D) is is very close to the ratio, 5.49, of the variances in $\hat{Z}_{num}$ observed in the simulations (Table 1).

These results paint a rather pessimistic picture of our ability to accurately remove the physical contribution from mortality estimates, especially for systems <50 km in extent

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(Fig. 2). Many existing mortality estimates thus would appear to be severely contaminated by physical effects. Ultimately, however, verification of a theory with simulated data is unsatisfying, and we turn, therefore, in the next section to a comparison made with real data.

Application to field data

In this section, we apply the HPI theory to field data to see if its predictions are valid for an uncontrolled setting and to demonstrate how the theory can be applied in practice, even with incomplete data. We reconsider an earlier study of ichthyoplankton mortality (Pepin et al. 1995) and focus attention directly on the errors associated in estimating transport because these were clearly dominant in the simulations and because fluxes were not discussed in any detail in HPI.

The leading term in the expression for the variance of $\hat{Z}_{\text{flux}}$ (HPI, eq. 44) is

$$(9)\qquad \text{var}(\hat{Z}_{\text{flux}}) \approx \left(\frac{L_x}{A\hat{C}_o}\right)^2 \text{var}(\hat{\Psi})$$

$$= \left(\frac{L_x}{A\hat{C}_o}\right)^2 \left\{ \int S_v(k_x,\omega)\,|\hat{F}_C(k_x,\omega)\hat{M}(\omega)|^2\,dk_x d\omega \qquad [\mathbf{T_1}] \right.$$

$$+ \int S_c(k_x,\omega)\,|\hat{F}_V(k_x,\omega)\hat{M}(\omega)|^2\,dk_x d\omega \qquad [\mathbf{T_2}]$$

$$+ 2\int S_{vc}(k_x,\omega)\,\hat{F}_C^{*}(k_x,\omega)\hat{F}_V(k_x,\omega)\,|\hat{M}(\omega)|^2\,dk_x d\omega \qquad [\mathbf{T_3}]$$

$$+ \left(\sum_{m=1}^{M}\left(\frac{\Delta l_m}{L_x}\right)^2 m^2(\delta t_m)v_{om}^2\right)\left(\sum_{n=1}^{N}\left(\frac{\Delta t_n}{\Delta T}\right)^2 m^2(T_{fn})\right)\sigma_c^2 \qquad [\mathbf{T_4}]$$

$$\left. + \left(\sum_{m=1}^{M}\left(\frac{\Delta l_m}{L_x}\right) m(\delta t_m)c_{om}\right)^2 \hat{M}^2(0)\sigma_u^2 \right\} \qquad [\mathbf{T_5}]$$

$$= \left(\frac{L_x}{A\hat{C}_o}\right)^2 \{\mathbf{T_1} + \mathbf{T_2} + \mathbf{T_3} + \mathbf{T_4} + \mathbf{T_5}\}$$

where

$$(10)\qquad \hat{F}_C(k_x,\omega) = \sum_{m=1}^{M}\left(\frac{\Delta l_m}{L_x}\right) m(\delta t_m)c_{om}e^{ik_x x_m}e^{-i\omega\delta t_m}$$

$$(11)\qquad \hat{F}_V(k_x,\omega) = \sum_{m=1}^{M}\left(\frac{\Delta l_m}{L_x}\right) m(\delta t_m)v_{om}e^{ik_x x_m}e^{-i\omega\delta t_m}$$

$$(12)\qquad \hat{M}(\omega) = \sum_{n=1}^{N}\left(\frac{\Delta t_n}{\Delta T}\right) m(T_{fn})e^{-i\omega T_{fn}}$$

All of the notation coincides with that used in HPI, but we note that L_x and L_y are the bay width and length, respectively, $A = L_xL_y$ is the surface area, ΔT is the time between full-bay surveys, $\hat{\Psi}$ is the estimated flux and $\hat{\Psi}_0$ is its mean value, $\hat{C}_o$ is the estimated basin mean plankton concentration averaged over ΔT, S_v and S_c are the velocity and plankton concentration autospectra and S_{vc} is the cross-spectrum, $\hat{F}_C$ and $\hat{F}_V$ are the respective discrete Fourier transforms of the mean larval concentration c_o and the mean out-bay current v_o, $\hat{M}$ is the discrete Fourier transform of the mortality function $m(t) = e^{-Z_e t}$ defined in terms of the effective mortality, M is the number of stations occupied during a flux transect, N is the number of flux transects executed during the experiment, Δl_m is the length interval along the flux transect line encompassing the mth station at $x = x_m$, δt_m is the time interval between adjacent stations along the flux transect line, T_{fn} is the central time of the nth flux transect and Δt_n is the time interval over which that transect applies, and σ_c^2 and σ_u^2 measure the variance in larval concentration and current speed due to experimental error.

As indicated by HPI, estimation of the various terms in eq. 9 requires knowledge of (*i*) the basin geometry and sampling plan, L_x, L_y, ΔT, N, M, x_m, δt_m, and T_{fn}, (*ii*) a first-order guess at the effective mortality, Z_e, (*iii*) the mean plankton and along-channel velocity along the open boundary, $c_o(x, L_y)$ and $v_o(x, L_y)$, (*iv*) the auto- and cross-spectra, $S_c(k_x, \omega)$, $S_v(k_x, \omega)$, and $S_{vc}(k_x, \omega)$, and (*v*) the experimental errors, σ_c and σ_u.

Before showing how each of these variables was calculated, we indicate how the various integrals in eq. 9 can be computed. Consider T_1 as an example. The integral can be approximated as a double summation over wave number and frequency if we specify an appropriate set for each. Given a wave number range of interest of $k_l \le k_x \le k_h$, we define the set $k_i = k_l + (i - 1)\Delta k$, $i = 1, N_k$ with $\Delta k = (k_h - k_l)/(N_k - 1)$. A similar choice is made for ω so that

$$(13)\qquad \mathbf{T_1} \approx \Delta k\Delta\omega\sum_{i=1}^{N_k}\sum_{j=1}^{N_\omega} S_v(k_i,\omega_j)\left|\hat{F}_C(k_i,\omega_j)\hat{M}(\omega_j)\right|^2.$$

In order to evaluate eq. 13, one must know S_v, $\hat{F}_C$, and $\hat{M}$ at the various k_i, ω_j points. It is thus advantageous to have simple analytical forms for these functions so that they can be computed for arbitrary k_x and ω. We therefore fit simple polynomial curves to the observations.

The field data come from multispecies ichthyoplankton surveys of Conception Bay carried out over 2- to 3-week periods in 1990 and 1991 (Pepin et al. 1995). All the basin and sampling plan variables are listed in Table 2, which reveals significant differences in execution between the two years. In 1990, biological and physical measurements were made from a single vessel. Within-basin ichthyoplankton surveys were conducted every 6–7 days (ΔT was chosen here as the time between the first and last surveys). On intervening days, transects were completed across the L_x = 20 km bay mouth every 5–6 days to monitor fluxes (the relatively long time gaps were due to weather and a ship failure). Ichthyoplankton tows were made at M = 7 stations, and currents were measured continuously along the transect with a 153-kHz boom-mounted ADCP. In 1991, the weather was significantly calmer and two ships were used, one for ichthyoplankton tows and the other for physical measurements. Within-bay and flux surveys were made every 6 and 1–2 days, respectively. Currents were again measured with an ADCP but using an improved, hydrodynamically smooth boom (Colbourne et al. 1993). Further survey details are contained in Pepin et al. (1995).

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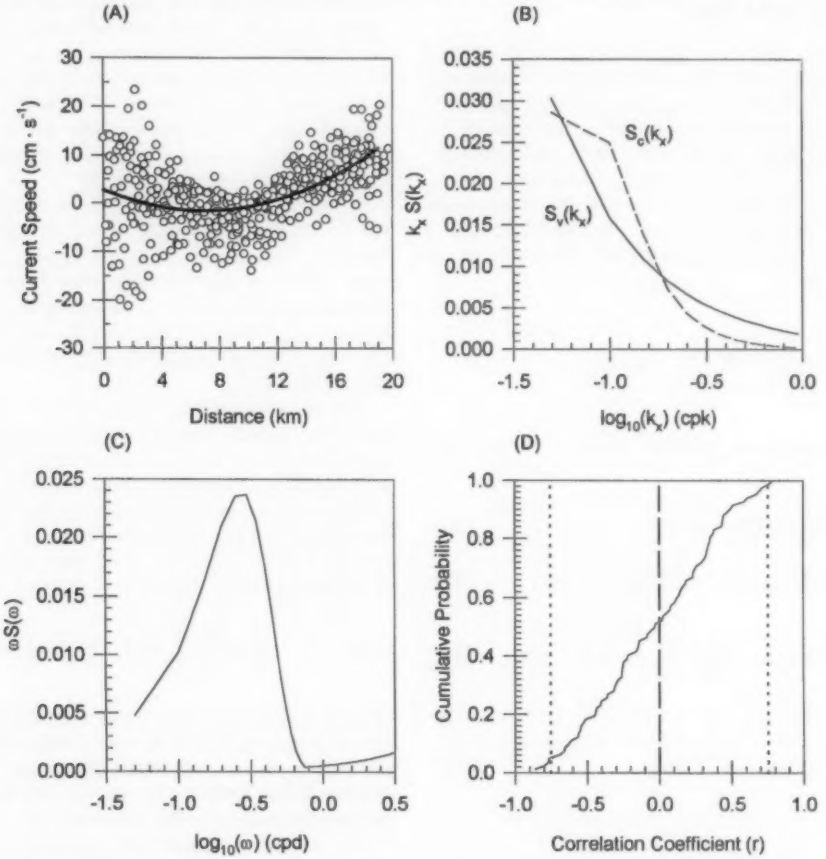
Fig. 6. (A) Scatterplot of the along-bay current across the mouth measured on 16 ADCP transects together with the best fit quadratic representing the mean; (B) wave number spectrum for currents (S_v) (solid line) and larval abundance (S_c) (dashed line); (C) average frequency spectrum computed for three current meters moored in Conception Bay (B. deYoung, Memorial University, St. John's, Nfld., personal communication); (D) cumulative probability distribution of the correlation coefficient (r) of larval concentration and currents measured for each occupation of the transect at the mouth of the bay. The dotted vertical lines show the values required to obtain a significant correlation ($p < 0.05$) based on a sample of seven stations. cpk, cycles·km^{-1}; cpd, cycles·day^{-1}.



The mean component of the along-bay current across the mouth and the spatial part of the along-bay current spectrum were estimated from 16 ADCP transects across the mouth. The mean was reasonably fit by a quadratic (Fig. 6A). A raw wave number spectrum was computed from the discrete Fourier transforms of the residuals for each transect, and the set of raw spectra were well fit by the curve $Ak^{-1.9}$ (Fig. 6B). However, the transects did not provide enough samples in time to permit computation of the temporal part of the spectrum. Consequently, we used the average spectrum computed for three current meters moored at 20 m depth for 6 months in Conception Bay (B. deYoung, Memorial University, St. John's, Nfld., personal communication), which shows a quadratic dependence on ω (Fig. 6C) and is broadly peaked in the weather band about a period of approximately 7 days. We then assumed that the full wave number – frequency spectrum was adequately represented by the product of the separately estimated parts.

There were six species present in sufficient numbers in each of the two years to allow estimation of their transport (one more than reported for 1990 by Pepin et al. 1995 (*Mallotus villosus*)). According to our previous findings

Table 2. Basin and sampling plan variables for the study by Pepin et al. (1995).

	1990	1991
L_x (km)	20	20
L_y (km)	50	50
ΔT (days)	17.5	10
N	4	8
T_{fn} (days)	−1.5, 4.5, 13.5, 16.5	0.5, 1.5, 2.5, 3.5, 6.5, 7.5, 8.5, 9.5
Δt_n	1.5, 7.5, 6.0, 2.5	1.0, 1.0, 1.0, 2.0, 2.0, 1.0, 1.0, 1.0
M	7	7
Δl_m (km)	2.7	2.7
δt_m (min)	43	22
J	13	9

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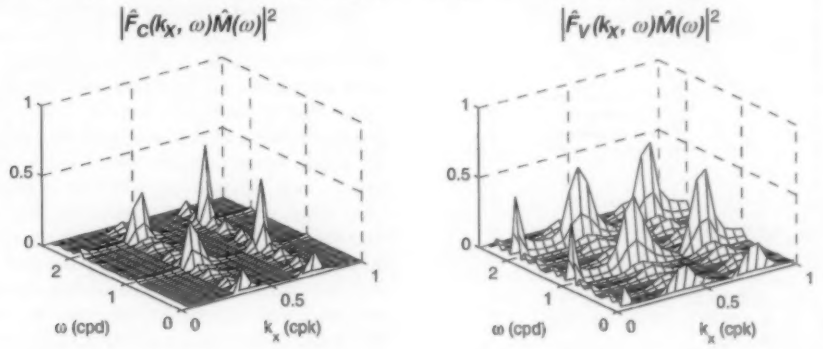
Table 3. Mean and range (in parentheses) of the variance components of total error in flux estimates for the study by Pepin et al. (1995).

Term	1990 variance (10^{-14}·s^{-2})	%	1991 variance (10^{-14}·s^{-2})	%
$\mathbf{T}$	3.74 (1.92–5.65)	—	3.26 (2.39–4.65)	—
$\mathbf{T}_1$	0.837	33 (16–57)	1.06	36 (25–44)
$\mathbf{T}_2$	1.33	37 (1–72)	1.02	30 (10–51)
$\mathbf{T}_3$	0	0		0
$\mathbf{T}_4$	0.967	24 (1–60)	0.364	11 (3–20)
$\mathbf{T}_5$	0.428	17 (8–30)	0.815	28 (19–35)

Note: Columns 2 and 4 report the overall mean for each variance component as well as the range in total variance. Columns 3 and 5 present the mean percentage and range of each variance component based on the estimate for each species included in the analysis.

Fig. 7. Perspective plots of $|\hat{F}_C(k_x, \omega)\hat{M}(\omega)|^2$ and $|\hat{F}_V(k_x, \omega)\hat{M}(\omega)|^2$. cpk, cycles·km^{-1}; cpd, cycles·day^{-1}.



(Pepin et al. 1995), each species/cohort combination could be treated as an independent realization of the effect of transport on population dynamics. Mean larval fields across the mouth showed no evidence of any consistent spatial patterns for any of the species/cohorts, and the mean field for each was thus considered to be spatially invariant. Mean concentrations ranged from 0.48 to 86 larvae·1000 m^{-3}. The spatial part of the larval spectrum was computed as the Fourier transform of the autocorrelation function of the 12 cohorts considered in this analysis augmented with data on 20 other species/cohorts sampled in 1990, 1991, and 1994 (P. Pepin, unpublished data) over station separations of 3–30 km. The analysis was not limited to the bay mouth, but used data from within the bay as well, and thus effectively assumed the larval distribution to be isotropic. We have no evidence to the contrary. On a log–log scale, $S_c(k_x)$ exhibits two slopes, –1.2 on scales between 7 and 30 km and –3.2 on smaller scales (Fig. 6B), and thus differs significantly from the velocity spectrum. Again, appropriate data did not exist to determine the temporal part of the spectrum, and we used that estimated for the current meters.

We approached the cross-spectral estimation in a similar manner by first estimating the cross-correlation between larval concentration and currents measured on the flux surveys for the 12 species considered here plus four others. The resulting correlations were normally distributed about 0, but only four of the 82 estimates were significantly different from 0 ($p < 0.05$) (Fig. 6D), so we concluded that there was no consistent association between larvae and currents and, hence, that $\mathbf{T}_3 \approx 0$.

We set Z_e equal to the values reported by Pepin et al. (1995), 0.10·day^{-1} in 1990 and 0.05·day^{-1} in 1991. Sensitivity to this choice is explored later. The variance in currents due to measurement error, σ_u^2, was set at $(0.007\ \text{m·s}^{-1})^2$ for 1990 and $(0.006\ \text{m·s}^{-1})^2$ for 1991 (Colbourne et al. 1993). The corresponding measurement error in the plankton hauls was estimated using the relationship given by Pepin and Shears (1997), $\sigma_c^2 = 3.2c_o^{3/2}$, for concentrations measured in units of number per cubic metre.

Each term in eq. 9 is species specific in that it depends on either the mean concentration or S_c. If we account for this dependence explicitly, for example by putting $S_c(k_x, \omega) = \text{var}(c)S_c'(k_x, \omega)$ and $\mathbf{T}_1 = \text{var}(c)\mathbf{T}_1'$, then eq. 9 can be rewritten as

$$\text{(14)}\quad \mathbf{T} = \left(\frac{L_x}{A}\right)^2 (\mathbf{T}_1' + V_c^2\mathbf{T}_2' + V_c\mathbf{T}_3' + c_o^{-1/2}\mathbf{T}_4' + \mathbf{T}_5' + c_o^{-1/2}\mathbf{T}_6')$$

where $V_c = \sqrt{\text{var}(c)}/c_o$ is the coefficient of variation. The primed terms are all species independent and need be computed only once. The V_c were computed from the transect data alone after first correcting the observed variance for sampling error, i.e., variance = observed variance – σ_c^2. The results are summarized in Table 3. Both $\mathbf{T}_1$ and $\mathbf{T}_2$ contribute a similar proportion of the average overall error in fluxes and together account for more than 65% of the predicted variance. Measurement error ($\mathbf{T}_4$ and $\mathbf{T}_5$) accounted for only about 30% of the overall variability. This is considerably higher than the value found in the simulations, but is consistent with the larger values of σ_c and σ_u.

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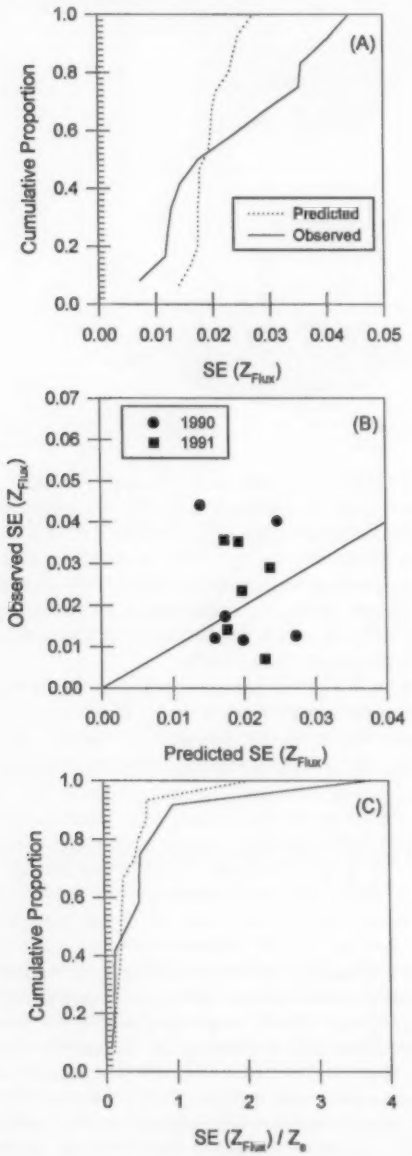
Fig. 8. (A) Cumulative proportion of predicted (dotted line) and observed (solid line) standard errors in the estimated flux based on the characteristics of Pepin et al.'s (1995) study.
(B) Scatterplot of the observed and estimated errors in estimated fluxes for the 12 individual species/cohorts that were part of the study. The solid line shows the one-to-one relationship.
(C) Cumulative proportion of predicted (dotted line) and observed (solid line) standard errors in the estimated flux standardized relative to the estimated mortality rate for individual species/cohorts. Standard errors are reported in units·day^{-1}.



Equation 9 shows that in order for $\mathbf{T_1}$ to be significant, the space and time scales selected by $|\hat{F}_C\hat{M}|^2$ must overlap the energetic part of the velocity spectrum S_v. Similarly, if $\mathbf{T_2}$ is to be important, then $|\hat{F}_V\hat{M}|^2$ and S_c must coincide. In the present example, c_o is essentially white, and thus, where one samples along the transect should not matter. By contrast, v_o is parabolic and how it is sampled should be important. These expectations are confirmed in the perspective plots of Fig. 7 which show that $|\hat{F}_C\hat{M}|^2$ is generally much smaller than $|\hat{F}_V\hat{M}|^2$. One would expect, therefore, $\mathbf{T_2}$ to be larger than $\mathbf{T_1}$. That the two terms are nearly the same size is due to fact that the velocity field is relatively more energetic than the larval field. (Specifically, $\mathbf{T_1}/\mathbf{T_2}$ is proportional to the ratio of the coefficients of variation for currents and concentration, and the former is about twice the latter.) The location and periodicity of the peaks, as explained in HPI, are determined by the sample spacing; the widths of the peaks depend on the spatial structure of v_o and c_o.

The predicted variance in estimated fluxes ranges from 1.46×10^{-14} to 5.36×10^{-14}·s^{-2} for the species considered in this analysis (Table 3). When compared with the observed error in estimated fluxes, HPI's model gives a distribution of values that is consistent with the observations, although the average is somewhat lower than the observations (Fig. 8A). On a one-to-one species/cohort basis, individual estimates display little relationship with one another (Fig. 8B). However, when normalized relative to the estimated mortality rates for each species, there is a close correspondence in the distribution of observed and predicted values (Fig. 8C). Probably the most important finding is that, on average, the predicted and observed variances are remarkably similar: HPI predicts the correct order of magnitude.

The comparison of observed and predicted values of the estimated error in the fluxes raises important issues. First, we should not expect a close one-to-one relationship. Each variance estimate made from the field data is, in effect, a single realization of a stochastic process which need not be near the average value given by the theory. In this example, predicted estimates of the flux variance were strictly based on the overall average means and variance. The only elements that differed in our estimates of var$\hat{\Psi}$ among the species/cohorts sampled in 1990 and 1991 were the coefficients of variation of the larva concentration estimated from the surveys whereas the field-derived values used the observed plankton and current measurements at each station. Second, any individual estimate of mortality correction due to transport must be interpreted in terms of its expected error. Our results suggest that this error is usually large and that the only practical way to reduce it is by obtaining a sufficient number of independent samples. We were fortunate in Pepin et al. (1995) in that each survey sampled several species simultaneously, thus allowing us to obtain samples from several realizations of the same underlying physical process. This yielded quasi-independent estimates of the underlying imprecision of our estimates of the fluxes.

Of course, the theoretical predictions are of value only if they do not depend too strongly on the parameters describing the environmental variability. To test the sensitivity of the predictions, we computed $\mathbf{T}$ for several values of the important parameters (Fig. 9). The greatest sensitivity was to the choice for the effective mortality, as $\mathbf{T}$ ranged between values 30% less to 25% more than the values reported here for values of Z_e one half and twice that assumed. $\mathbf{T}$ showed little sensitivity to variations of the slope of S_v and moderate

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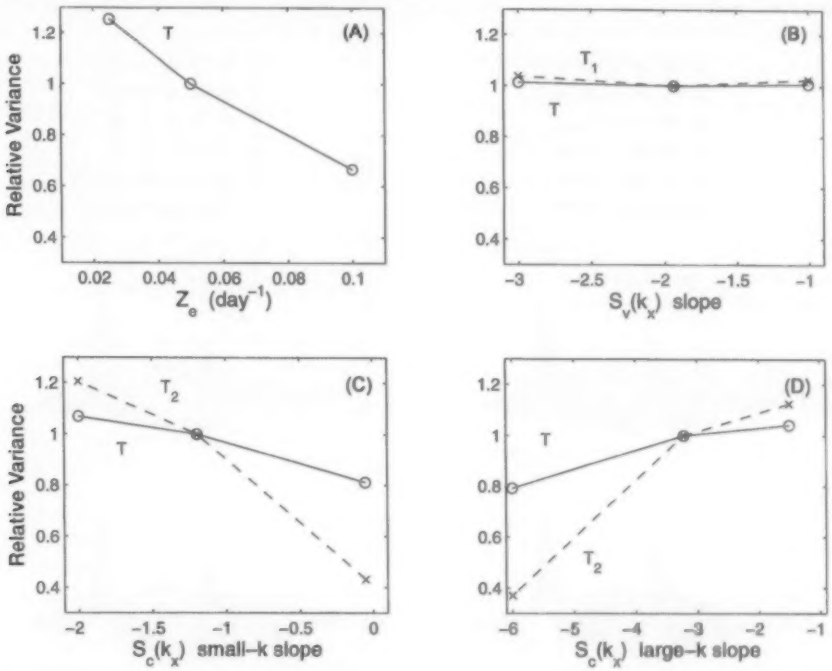
Fig. 9. Sensitivity of **T** to (A) Z_e; (B) slope of the velocity wave number spectrum; (C) low wave number slope of the plankton spectrum; and (D) high wave number slope of the plankton spectrum.



dependence on the slope of S_c. Not shown are the dependencies on the variances of the current and plankton fields or the measurement errors. Since $\mathbf{T_1}$ and $\mathbf{T_2}$ are directly proportional to var(v) and var(c), respectively, variations in either would yield a similar sensitivity to that found for Z_e. Sensitivity to measurement error is also strictly proportional. The relatively small range for each parameter demonstrates that the estimates are reasonably robust and can be applied with some confidence. Individual components of **T** vary more widely, but the overall effect on **T** is relatively uniform.

Array design

We have referred several times here and in HPI to the potential use of the theory in a priori array design for ichthyoplankton experiments. Array design is a difficult subject that is the focus of an ongoing study that is attempting to link the present theory with other approaches. Our only purpose here is to demonstrate some of the theory's potential.

To do so, we return to the example considered in HPI of the estimation of the single-survey within-bay plankton concentration for a 20 × 20 km basin using a 36-station, 24-h sampling scheme. The variability in estimated concentration is given by (HPI, eq. 28)

$$(15) \quad \mathrm{var}(\hat{C}_T) = m^2(T) \int S_c(\mathbf{k}, \omega) \left| \hat{H}(\mathbf{k}, \omega) \right|^2 \mathrm{d}\mathbf{k}\mathrm{d}\omega$$

where T is the central time of the survey. HPI displayed two-dimensional slices of $|\hat{H}|^2$ for an array in which the stations were occupied row-wise (Fig. 10A). A comparison of the dependence on the cross-bay wave number k_x against that on the along-bay wave number k_y (Figs. 10B and 10C) revealed a dramatic asymmetry that originates in the row-wise strategy. For contrast, we show two-dimensional slices here for a sampling plan that uses a spiral-like pattern (Figs. 10D–10F). In terms of the total distance and total time required, the two sampling plans are identical. Yet, $|\hat{H}|^2$ is much more nearly isotropic for the spiral plan, and the area under the curve in Fig. 10F is only about 60% that of Fig. 10C (for length scales <20 km and time scales <25 days). The implication is not necessarily that the spiral plan is a better one, but that fairly minor differences in design can result in major differences in sensitivity to environmental variability. The spiral plan is only better if $|\hat{H}|^2$ is large where S_c is not. Lacking some prior knowledge of basin dynamics, this information provided through $|\hat{H}|^2$ would be difficult to exploit. However, the necessary information might be inferred from factors as simple as basin shape. In any case, the experimenter should possess some understanding of the characteristics of the sampling plan prior to executing a field study.

Discussion

The theory developed by HPI partitions the bias and variance of mortality and flux estimates according to the space and time scales of the underlying current and larval fields. We validated this theory with an extensive set of numerical experiments as well as with field data from a previous study. The model performed well in the simulations, where all environmental variability is known exactly, as well as for field

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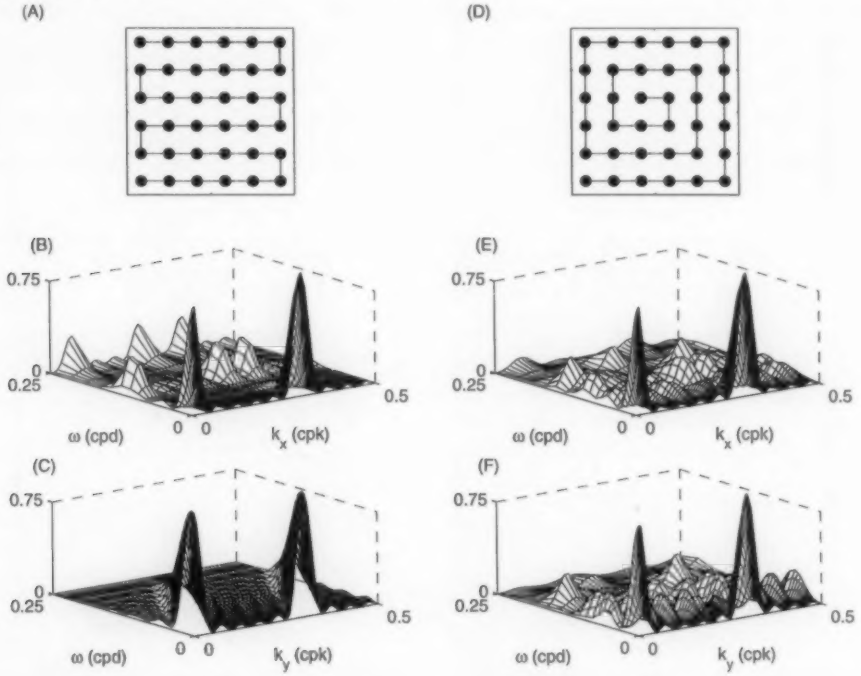
Fig. 10. Comparison of two sampling arrays. (A) Row-wise sampling plan; (B) $|\hat{H}(k_x, k_y = 0, \omega)|^2$ for the row-wise plan; (C) $|\hat{H}(k_x = 0, k_y, \omega)|^2$ for the row-wise plan; (D) spiral plan; (E) $|\hat{H}(k_x, k_y = 0, \omega)|^2$ for the spiral plan; (F) $|\hat{H}(k_x = 0, k_y, \omega)|^2$ for the spiral plan. cpk, cycles·km^{-1}; cpd, cycles·day^{-1}.



conditions, where environmental conditions could only be estimated. This implies that the theory can be applied in practice with reasonable confidence.

Accurate measurement of mortality (and growth) rates is fundamental to determining the factors that influence the dynamics of the early life stages of fish which appear to be important to ultimately determining their relative year-class strengths. For example, accurate knowledge of these rates is vital in understanding the role of predator–prey interactions in regulating egg and larval survival (Bailey and Houde 1989). The present results imply that the expected error in the apparent mortality, $\hat{Z}_{num}$, is about 20% for many systems, indicating that useful estimates of the effective mortality can be made in practice. Whether or not Z_e is close to Z depends on transport. Our comparison with field data showed that individual flux estimates were about 50% of the overall mortality estimates, and we thus conclude that many mortality estimates should be corrected for transport effects. However, the large error in the flux estimates suggests a limited ability to do so unless a sufficient number of independent flux estimates are obtained. The most obvious solution is to measure the mortalities of several independent species simultaneously. In fact, it was only by doing so that Pepin et al. (1995) were able to obtain meaningful results.

Our results thus imply that many previous estimates of egg and larval mortality rates are likely contaminated by transport effects, especially for systems <50 km in extent. Although this has been previously suggested (Taggart and Frank 1990; Pepin et al. 1995; HPI), the support for this hypothesis is now much stronger. Ultimately, this raises questions about earlier inferences concerning the role of biotic processes in regulating ichthyoplankton mortality rates (Peterson and Wroblewski 1984; McGurk 1986; Houde 1989; Pepin 1991).

Ichthyoplankton survey designs often adopt either systematic (e.g., Smith and Richardson 1977) or stratified approaches (e.g., Sherman et al. 1984) and generally implicitly assume that the populations can be sampled synoptically within the scope of each survey. The present study demonstrates the fragility of this assumption. Repeated estimates of larval fish mortality rates from Zweifel and Smith (1981), Lo (1985), and Taggart and Leggett (1987) suggest that the relative variance in effective mortality Z_e ranges from 20 to 100% among cohorts and is greater in small systems in consonance with the results presented here. The present study suggests that it may be difficult, if not infeasible, to determine the sources of that variation simply because of the difficulties in accurately sampling populations that are highly dynamic in both space and time.

Cushing (1975), Sissenwine (1984), and Myers and Cadigan (1993) have suggested that variability in the survival of fish during the early life history is the primary cause of variations in year-class strength. In general, few studies have been able to observe catastrophic events (Leggett and Deblois 1994), suggesting that the length of time that eggs and larvae spend in a stage vulnerable to high mortality has a direct impact on overall survival (McGurk 1986; Houde 1989; Pepin 1991). Under such circumstances, small changes in either growth or mortality rates can generate substantial variation in year-class strength (Houde 1987; Beyer

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1989). If this hypothesis is correct, then the results of our study indicate that it is unlikely that attempts at measuring larval mortality rates will provide the accuracy and precision required to understand the processes regulating survival. Under some circumstances, broad-scale surveys of highly abundant species may provide adequate representation of the magnitude in estimated mortality rates with limited bias and variance (e.g., Lo et al. 1989; recall that $\text{var}(\hat{Z}_{\text{num}})$ is inversely proportional to $\hat{C}_{oI}^2$). However, even if it is possible to correct changes in abundance for losses attributable to physical processes, variations in estimated mortality rates caused by sampling variability alone (i.e., their coefficient of variation) may be of the same order as the changes required to cause substantial variations in year-class strength. The only alternative may be to develop studies using multispecies sampling strategies. One could then ascertain if estimated vital rates differ significantly among species. Failure to reject the null hypothesis may then allow us to use each species as an independent sample of a single process (Pepin et al. 1995). Two important points of interpretation must be seriously considered. First, failure to measure significant differences among species may be due simply to inappropriate survey design, a possibility that must be seriously explored. Alternatively, similarity of vital rates may reflect vulnerability to similar environmental conditions and would thus suggest a degree of synchrony among species.

Oceanographers and biologists may be most capable of estimating the average vital rates of a population (i.e., mean mortality and growth rates). As a result, our principle research objectives may need to focus on the underlying factors and processes that govern average rates of growth and loss of populations of fish eggs and larvae rather than the fluctuations about the mean. Ichthyoplankton surveys have been used to estimate adult biomass, determine spawning locations, and describe the basic life cycle and environmental vulnerability of marine fishes. For example, supply of larvae to suitable habitats (e.g., nursery areas) has been proposed as the underlying process of greatest importance in determining recruitment variability in a number of species (Doherty and Williams 1988; Roughgarden et al. 1988; Underwood and Fairweather 1989). Understanding the factors that determine the general variability in supply and distribution and postsettlement or aggregative processes may prove to be a fundamental requirement for understanding the regulation of variations in year-class strength in marine fish populations.

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References

Bailey, K.M., and Houde, E.D. 1989. Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. **25**: 1–83.

Bennett, A.F., and Denman, K.L. 1985. Phytoplankton patchiness: inferences from particle statistics. J. Mar. Res. **43**: 307–335.

Beyer, J. 1989. Recruitment stability and survival — simple size-specific theory with examples from the early life dynamics of marine fish. Dana Rep. **7**: 45–147.

Colbourne, E.B., Helbig, J.A., and Cumming, D. 1993. Improved ADCP performance using a hydrodynamically designed boom mount. J. Atmos. Oceanic Technol. **10**: 629–636.

Cushing, D.H. 1975. Marine ecology and fisheries. Cambridge University Press, Cambridge, U.K.

deYoung, B., and Sanderson, B. 1995. The circulation and hydrography of Conception Bay, Newfoundland. Atmos.-Ocean, **33**: 135–162.

Doherty, P.J., and Williams, D. 1988. The replenishment of coral reef fish populations. Oceanogr. Mar. Biol. Annu. Rev **26**: 487–551.

Firing, E. 1993. Structure of the upper ocean velocity field on scales larger than 10 kilometers. *In* Proceedings 'Aha Huliko'a. *Edited by* P. Muller and D. Henderson. University of Hawaii at Manoa. pp. 37–54.

Haidvogel, D.B., Wilkin, J.L., and Young, R. 1991. A semi-spectral primitive equation ocean circulation model using vertical sigma and orthogonal curvilinear horizontal coordinates. Methods Comput. Phys. **94**: 151–185.

Helbig, J.A., and Pepin, P. 1998. Partitioning the influence of physical processes on the estimation of ichthyoplankton mortality rates. I. Theory. Can. J. Fish. Aquat. Sci. **55**. This issue.

Houde, E.D. 1987. Early life dynamics and recruitment variability. Am. Fish. Soc. Symp. **2**: 17–29.

Houde, E.D. 1989. Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fish. Bull. U.S. **87**: 471–495.

Ledwell, J.R., Watson, A.J., and Law, C.S. 1993. Evidence for slow mixing across the pycnocline from an open-ocean tracer-release experiment. Nature (Lond.), **364**: 701–703.

Leggett, W.C., and Deblois, E. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Neth. J. Sea Res. **32**: 119–134.

Lo, N.C.H. 1985. Egg production of the central stock of northern anchovy 1951–1983. Fish. Bull. U.S. **83**: 137–150.

Lo, N.C.H., Hunter, J.R., and Hewitt, R.P. 1989. Precision and bias of estimates of larval mortality. Fish. Bull. U.S. **87**: 399–416.

McGurk, M.D. 1986. Natural mortality of marine pelagic fish eggs and larvae: role of spatial patchiness. Mar. Ecol. Prog. Ser. **34**: 227–242.

Monin, A.S., and Yaglom, A.M. 1975. Statistical fluid mechanics. Vol. 2. MIT Press, Cambridge, Mass.

Myers, R.A., and Cadigan, N.G. 1993. Density-dependent juvenile mortality in marine demersal fish. Can. J. Fish. Aquat. Sci. **50**: 1576–1590.

Pepin, P. 1991. Effect of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Can. J. Fish. Aquat. Sci. **48**: 503–518.

Pepin, P., and Shears, T.H. 1997. Variability and capture efficiency of bongo and Tucker trawl samplers in the collection of ichthyoplankton and other macrozooplankton. Can. J. Fish. Aquat. Sci. **54**: 765–773.

Pepin, P., Helbig, J.A., Laprise, R., Colbourne, E., and Shears, T.H. 1995. Variations in the contribution of transport to changes in planktonic animal abundance: a study of the flux of fish larvae in Conception Bay, Newfoundland. Can. J. Fish. Aquat. Sci. **52**: 1475–1486.

Peterson, I., and Wroblewski, J.S. 1984. Mortality rates of pelagic

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fishes in the pelagic ecosystem. Can. J. Fish. Aquat. Sci. **41**: 1117–1120.

Roache, P.J. 1976. Computational fluid dynamics. Hermosa, Albuquerque, N.M.

Roughgarden, J., Gaines, S., and Possingham, H. 1988. Recruitment dynamics in complex life cycles. Science (Washington, D.C), **241**: 1460–1466.

Sherman, K., Smith, W., Morse, W.W., Berman, M., Green, J., and Ejsymont, J. 1984. Spawning strategies of fishes in relation to circulation, phytoplankton production, and pulses of zooplankton off the northeastern United States. Mar. Ecol. Prog. Ser. **18**: 1–19.

Sissenwine, M.P. 1984. Why do fish populations vary? *In* Exploitation of marine communities. *Edited by* R.M. May. Springer-Verlag, Berlin. pp. 59–94.

Smith, P.C., and Schwing, F.B. 1991. Mean circulation and variability on the eastern Canadian continental shelf. Continental Shelf Res. **11**: 977–1012.

Smith, P.E., and Richardson, S.L. 1977. Standard techniques for pelagic egg and larval surveys. FAO Fish. Tech. Circ. No. 175.

Taggart, C.T., and Frank, K.T. 1990. Perspectives on larval fish ecology and recruitment processes: probing the scales of relationships. *In* Large marine ecosystems: patterns, processes, and yields. *Edited by* K. Sherman, L.M. Alexander, and B.D. Gold. American Association for the Advancement of Science, Washington, D.C. pp. 151–164.

Taggart, C.T., and Leggett, W.C. 1987. Short-term mortality in postemergent capelin *Mallotus villosus*. I. Analysis of multiple in situ estimates. Mar. Ecol. Prog. Ser. **41**: 205–217.

Underwood, A.J., and Fairweather, P.G. 1989. Supply-side ecology and benthic marine assemblages. Trends Ecol. Evol. **4**: 16–20.

Weaver, A.J., and Sarachik, E.S. 1990. On the importance of vertical resolution in certain ocean general circulation models. J. Phys. Oceanogr. **20**: 600–609.

Zweifel, J.R., and Smith, P.E. 1981. Estimates of abundance and mortality of larval anchovies (1951–75): application of a new method. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **178**: 248–259.

Appendix. Specification of the velocity field

Each velocity component takes the form

$$(A1)\quad u(x,y,t) = \sum_{k=-N_k}^{N_k}\sum_{l=-N_k}^{N_k}\sum_{m=-N_\omega}^{N_\omega} a_{klm} \times \cos(k_k x + k_l y + \omega_m t + \phi_{klm})$$

where N_k and N_ω are the number of wave numbers and frequencies included in the model (30 and 40, respectively) and the ϕ_{klm} are random phases drawn from a uniform distribution on $[0,2\pi]$ at the beginning of the simulation. The coefficient a_{klm} is defined by

$$(A2)\quad a_{klm} = [S_u(k_k, k_l, \omega_m)\Delta k_k \Delta k_l \Delta\omega_m]^{1/2}$$

where Δk and $\Delta\omega$ are spacings between adjacent wave number and frequency components. Note that the sums run over negative wave numbers and frequencies as well as positive ones in order to admit constituent waves of all possible propagation directions. Note also that $a_{klm} = 0$ for k, l, or $m = 0$, so there is no mean component.

For gridpoint (i, j), the dependence on wave number can be separated from that on frequency to give

$$(A3)\quad u_{ij}(t) = \sum_{m=1}^{N_\omega} A_{ijm}\cos(\omega_m t) - B_{ijm}\sin(\omega_m t)$$

where

$$(A4)\quad A_{ijm} = \sum_{k=1}^{N_k}\sum_{l=1}^{N_k} a_{klm} \{\cos(k_k x_i + k_l y_j)(c_{+++} + c_{++-} + c_{--+} + c_{---}) - \sin(k_k x_i + k_l y_j)(s_{+++} + s_{++-} - s_{--+} - s_{---}) + \cos(k_k x_i + k_l y_j)(c_{-++} + c_{+-+} + c_{+++} + c_{+--}) - \sin(k_k x_i - k_l y_j)(s_{-++} + s_{+-+} - s_{+++} - s_{+--})\}.$$

The c and s terms are cosines and sines of the random phases, e.g., $(c_{+-+} = \cos(\phi_{k_x,-k_y,\omega})$ with appropriate choices of k_x, k_y, and ω dictated by the subscripts k, l, and m. A similar expression holds for B_{ijm}.

A_{ijm} and B_{ijm} were generated at the beginning of each run, and u_{ij} was updated at each time step using eq. A3. The vast majority of computer time was spent in the former task (about 95%), while the remainder proceeded very quickly.

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Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation

Peter M. Chapman, Feiyue Wang, Colin Janssen, Guido Persoone, and Herbert E. Allen

Abstract: Major metal-binding phases in the aerobic layer of sediments are iron and manganese oxyhydroxides (FeOOH and MnOOH) and particulate organic carbon (POC). The acid-volatile sulfide (AVS) model proposed for predicting nontoxicity from metals-contaminated sediments is only applicable to anaerobic sediments. In other sediments, normalization by POC or FeOOH and MnOOH may be predictive, but binding constants are not well understood. Metal mobilization is enhanced by ligand complexation and oxidation of anaerobic sediments. Free metal ion is the most bioavailable species, but other labile metal species and nonchemical variables also determine metal bioavailability; biotic site binding models have shown promise predicting toxicity for systems of differing chemistry. Hazard identification and ecological risk assessment (ERA) depend on determining bioavailability, from water (overlying, interstitial) and food, which can be done prospectively (e.g., normalized sediment chemistry, laboratory bioassays) or retrospectively (e.g., in situ bioassays, field studies). ERA of sediment-bound metals requires primary emphasis on toxicity and consideration of the three separate transformation processes for metals in the aquatic environment, the differences between essential and nonessential metals, the complex interactions that control bioavailability, adaptation, which may occur relatively simply without appreciable cost to the organism, weight of evidence, and causality.

Résumé : Les principales matières liant les métaux dans les couches de sédiments aérobies sont les oxyhydroxydes de fer et de manganèse (FeOOH et MnOOH) et le carbone organique particulaire (COP). Le modèle du sulfure volatil acide proposé pour prédire la non-toxicité de sédiments contaminés par des métaux ne s'applique qu'aux sédiments anaérobies. Dans d'autres sédiments, la normalisation par rapport au COP ou au FeOOH et MnOOH permet de faire des prévisions, mais les constantes de liaison sont mal connues. La formation de complexes avec des ligands et l'oxydation des sédiments anaérobies augmentent la mobilisation des métaux. L'ion métallique libre est la principale forme biodisponible, mais d'autres formes labiles de métaux le sont également et des variables non spécifiques ont aussi un rôle à jouer dans la biodisponibilité; des modèles de liaison à un site biotique se sont révélés prometteurs pour prédire la toxicité dans le cas de systèmes aux propriétés chimiques différentes. Pour déterminer les dangers et évaluer le risque écologique des métaux, il faut déterminer leur biodisponibilité à partir de l'eau (eau sus-jacente, interstitielle) et des aliments, ce que l'on peut faire de façon prospective (normalisation des paramètres chimiques des sédiments, épreuves biologiques en laboratoire) ou rétrospective (épreuves biologiques in situ, études sur le terrain). Pour évaluer le risque écologique des métaux liés aux sédiments, il faut surtout déterminer leur toxicité, étudier les trois processus différents de transformation des métaux dans le milieu aquatique et tenir compte des différences entre les métaux essentiels et non essentiels, des interactions complexes qui influent sur la biodisponibilité, de l'adaptation de l'organisme qui peut se produire relativement simplement et sans dommages notables, de la pertinence des données et des relations de cause à effet.

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Introduction

Sources of metals to sediments vary and include natural and anthropogenic sources, point and nonpoint sources, and spills. Determining whether sediments contaminated with metals and (or) organics can adversely affect biota (i.e., are polluted rather than simply contaminated) has been the focus of numerous publications, particularly since the early 1980s (reviewed by Chapman 1997*a*). Surprisingly, however, review papers have not examined sediment ecotoxicology holistically. Rather, they have primarily focused on toxicity testing methodology (Chapman 1988; Lamberson and Swartz 1988; Giesy and Hoke 1989; Burton and Scott 1992) with some additional reviews of general assessment meth-

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ods, including toxicity testing (Reynoldson and Zarull 1989; Adams et al. 1992).

The goal of this paper is to provide a synthesis of current science related to determining the hazard and risk posed by metals-contaminated sediment, what it means, and provide our opinions as to where the science should be going. We use the term "hazard" to mean a source of potential danger to the environment and the term "risk" to mean the probability that such hazard will be realized. We begin this review by discussing mechanisms of metals association with and release from sediments followed by bioavailability pathways, tools for hazard identification and ecological risk assessment (ERA), remediation, and then conclusions and research needs. The principles discussed herein are fundamentally similar for fresh, estuarine, and marine waters.

Mechanisms of metals binding to and release from sediments

Metal-binding phases in sediments

Acid-volatile sulfide (AVS)

The role of exchangeable iron monosulfides (FeS) and manganese monosulfides (MnS) in determining the bioavailability of trace metals in anaerobic sediments has been studied intensively (Ankley et al. 1996). Formed by reduction of sulfate both by direct bacterial reduction and indirectly as an electron acceptor in the bacterial oxidation of organic matter, a majority of sulfide in sediments is in the form of FeS (amorphous FeS, mackinawite, greigite, pyrrhotite, or troilite), MnS, iron bisulfide (pyrite), or organic sulfide (Morse et al. 1987; Di Toro et al. 1990), among which FeS and MnS are the most labile fractions and have been operationally defined as AVS (Morse et al. 1987). Since FeS and MnS have higher solubility products (K_{sp}) than all other trace metal sulfides (MeS), metals will displace Fe and Mn to form more insoluble MeS:

$$(1)\quad Me^{2+} + FeS(s) = MeS(s) + Fe^{2+}$$

$$(2)\quad Me^{2+} + MnS(s) = MeS(s) + Mn^{2+}.$$

The MeS formed can be either in discrete (pure) MeS phases or in a solid solution formed by coprecipitation with and (or) adsorption on FeS (Morse et al. 1987; Huerta-Diaz et al. 1998). The activity of MeS is equal to 1 when a pure MeS phase forms whereas far less than 1 when a solid solution forms according to both ideal and nonideal solid solution models. Hence, if all the metal in sediments is in the form of MeS, the free metal ion activity in interstitial waters will be controlled by dissolution of MeS:

$$(3)\quad MeS(s) + H^+ = Me^{2+} + HS^-, K_{sp,MeS}$$

$$(4)\quad \{Me^{2+}\} = K_{sp,MeS}\{MeS\}\{H^+\} / \{HS^-\} \le K_{sp,MeS}\{H^+\}/\{HS^-\}$$

where $\{I\}$ denotes the activity of species I.

In the presence of excess Fe, Di Toro et al. (1992) have shown the following inequality to be valid:

$$(5)\quad \{Me^{2+}\} / [M_T] < K_{sp,MeS} / K_{sp,FeS}.$$

Because the sulfide solubility products of the metals of environmental interest are far less than that of FeS, the activities of these metals will be very small in the presence of excess AVS. Consequently, if AVS is in excess, the metals are not likely toxic.

Particulate organic carbon (POC)

POC in sediments has a strong binding affinity for metals. Its contribution is particularly important in either aerobic sediments where no or little AVS is present (Fu et al. 1992; Tessier et al. 1993, 1996; Wang et al. 1997) or anaerobic sediments where trace metal concentrations are greater than the AVS (i.e., all the binding capacity of the AVS has been exhausted) (Mahony et al. 1996). Since nonhumic substances (such as carbohydrates, proteins, amino acids, fats, and resins) have a relatively short residence time (they are easily decomposed by microorganisms), the major component of POC in sediments is humic substances. Although the binding of metals to humic substances has been studied intensively, no theoretical model is yet available because of the complexity and diversity of humic substances in sediments. However, several general models, such as Langmuir adsorption (Mahony et al. 1996), surface complexation (Davis 1984; Tessier et al. 1993), and ion binding (Marinsky and Ephraim 1986), have been applied or developed to describe the binding of metals by humic substances. Humic Ion-Binding Model V (Tipping and Hurley 1992; Tipping 1994), an improved ion-binding model, although not yet fully tested, is probably the most reasonable and feasible. It assumes that metal binding can occur at monodentate or bidentate proton-dissociating sites of humic substances that can be described by combinations of intrinsic equilibrium constants and electrostatic terms:

$$(6)\quad \equiv RAH^Z + Me^{2+} = \equiv RAMe^{Z+1} + H^+, K_{HA\text{-}Me}$$

$$(7)\quad \{Me^{2+}\} = [\equiv RAMe^{Z+1}][H^+]e^{4wZ} / K_{HA\text{-}Me}[\equiv RAH^Z]$$

where Z is the charge on the humic molecule, w is an electrostatic interaction factor related to ionic strength, and $[I]$ denotes the concentration of species I.

Mahony et al. (1996) found that the binding of metals to sediment organic carbon (OC) can also be simply described by the Langmuir adsorption model:

$$(8)\quad Me^{2+} + POC = POC\text{-}Me, K_{OC\text{-}Me}$$

$$(9)\quad [POC\text{-}Me] = [POC\text{-}Me]^0 K_{OC\text{-}Me}\{Me^{2+}\}/([POC\text{-}Me]^0 + K_{OC\text{-}Me}/\{Me^{2+}\})$$

where [POC-Me] is the OC-normalized metal concentration bound by POC in sediments, $[POC\text{-}Me]^0$ is the sorption capacity, and $K_{OC\text{-}Me}$ is the OC-normalized partition coefficient. When $\{Me^{2+}\} << [POC\text{-}Me]^0/K_{OC\text{-}Me}$, the model reduces to linear partitioning:

$$(10)\quad \{Me^{2+}\} = [POC\text{-}Me] / K_{OC\text{-}Me}.$$

Iron and manganese oxyhydroxides (FeOOH and MnOOH)

In aerobic sediments, FeOOH and MnOOH, as well as POC, are the dominant binding phases of metals in sediments (Davis-Colley et al. 1984; Tessier et al. 1993, 1996). The adsorption of trace metals by pure iron and manganese oxides in aqueous solution has been well described by sur-

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face complexation (Dzombak and Morel 1990; Smith and Jenne 1991). Balistrieri et al. (1981) and Davis (1984) theorized that organic matter coated on the surfaces of FeOOH and MnOOH will mask the reactivity of the underlying oxides. Tessier et al. (1996) isolated diagenetic FeOOH and MnOOH from two geochemically different lake sediments. They found that masking of the oxides by organic matter does not always occur in nature; metal association with naturally occurring FeOOH and MnOOH was verified. In an acidic lake, trace metals were bound to coated organic matter. In a circumneutral lake, Cu, Pb, Zn, Cd, and Ni were directly bound by the hydroxyl groups of the underlying FeOOH and MnOOH and the measured conditional binding constants were in reasonable agreement with predicted constants from laboratory-derived surface complexation data sets. Therefore, the binding of trace metals to naturally occurring FeOOH and MnOOH can be generally described as the following surface complexation reactions:

$$(11) \quad \equiv \mathrm{FeOOH} + \mathrm{Me}^{2+} = \equiv \mathrm{FeOOMe}^{+} + \mathrm{H}^{+},\ K_{\equiv \mathrm{FEOOH,Me}}$$

$$(12) \quad \{\mathrm{Me}^{2+}\} = [\equiv \mathrm{FeOOMe}^{+}][\mathrm{H}^{+}]e^{\psi F/RT} / K_{\equiv \mathrm{FeOOH,Me}}[\equiv \mathrm{FeOOH}]$$

where ψ is the surface potential (volts), F is Faraday's constant, R is the molar gas constant (8.314 $\mathrm{J \cdot mol^{-1} \cdot K^{-1}}$), and T is the absolute temperature (kelvins).

Complexation by ligands in interstitial waters

Complexation of metals by soluble inorganic and organic ligands in waters enhances metal mobilization significantly. As a result, the soluble metal concentrations in interstitial waters are frequently higher than predicted on the basis of partitioning between sediment metal-binding phases and porewaters (Boulegue 1983; Huerta-Diaz et al. 1998). Similarly, ligands affect bioavailability (e.g., of Cu to bacterial cells and to chitin; Vasconcelos et al. 1997). As discussed later, it is important to understand the relationship between the free metal ion, other bioavailable metal species, and complexed metal ligands if porewater measurements are compared with water-only toxicity measurements made on the basis of total soluble metal concentration (e.g., interstitial water toxicity unit, interstitial water criteria toxicity unit).

Complexation of metals by $\mathrm{OH^-}$, $\mathrm{F^-}$, $\mathrm{Cl^-}$, $\mathrm{HCO_3^-}$, $\mathrm{SO_4^{2-}}$, $\mathrm{CO_3^{2-}}$, and $\mathrm{HPO_4^{2-}}$ has been well documented (Newman and Jagoe 1994). Complexation by humic substances can be potentially described by Humic Ion-Binding Model V (Tipping and Hurley 1992; Hare and Tessier 1996). Although metal complexes with sulfide and polysulfides probably dominate metal speciation in anoxic porewaters (Boulegue 1983; Huerta-Diaz et al. 1998), their formation constants are still poorly known, despite increasing attention in recent years (e.g., Luther and Ferdelman 1993; Zhang and Millero 1994; Luther et al. 1996; Paquette and Helz 1997). Similar attention has not been paid to the high affinity of thiols for metals, particularly the hydrophilic thiols with low molecular weights (such as cysteine, glutathione, 3-mercaptopropanoate, thioglycolic acid, and methanethiol). Such attention may be merited, since they have been found in detectable levels in porewaters (Shea and MacCrehan 1988; MacCrehan and Shea 1995).

Two key points arise from the above. First, complexation by ligands can enhance mobilization. Second, complexation has the potential to reduce bioavailability.

Oxidation caused by physical, biological, and human activities

Sediment–porewater equilibrium is typically assumed for simplicity. However, the surficial sediment zone is a dynamic chemical and biological system sensitive to redox potential. A small increase in redox potential can result in the oxidation of MeS and sulphide, enhancing the release of metals from sediments to porewaters and hence increasing the bioavailability of metals (Zhuang et al. 1994; Peterson et al. 1996). This is particularly important because surficial sediments contribute significantly to the exposure of benthic organisms to contaminants. Several processes can increase the redox potential in sediments: (*i*) temporal and spatial changes in rates of particle deposition and microbial reduction of sulfate, which result in the seasonal and spatial variation of AVS (e.g., Besser et al. 1996*a*; Hansen et al. 1996*a*; Liber et al. 1996), (*ii*) bioturbation and bioirrigation by the burrowing, feeding, tubing, excretion, respiration, and locomotion activities of benthic animals (Tessier et al. 1994; Matisoff 1995; Gagnon et al. 1996; Peterson et al. 1996), and (*iii*) sediment resuspension caused by storms, flooding, tidal exchanges or currents, dredging, and trawling.

Both dissolved sulphide and FeS (AVS) in sediments are oxidized rapidly (several hours) when exposed to $\mathrm{O_2}$ (e.g., Zhuang et al. 1994; Gonzalez 1996). A monodisperse particle surface oxidation model (Di Toro et al. 1996*a*) has been developed to described the oxidation of synthesized FeS and AVS:

$$(13) \quad c(t) = c(0)(1 - kt)^{\nu}$$

where $c(t)$ is the concentration (milligrams per litre) of FeS at time t and the dimensional exponent ν ($1 \leq \nu \leq 3$) characterizes particle morphology and may depend on pH, ionic strength, and dissolved $\mathrm{O_2}$. The oxidation rate constant k can be described by a surface complexation model and is essentially the same for synthesized FeS and for sediment AVS (Di Toro et al. 1996*a*). However, the model fails in high $\mathrm{O_2}$ (approaching 1 atm (101.325 kPa)) and at the end of oxidation (Di Toro et al. 1996*a*).

The general observed difference in stability between MeS (e.g., CdS, ZnS) and AVS with respect to oxidation (Liber et al. 1996; Peterson et al. 1996; Di Toro et al. 1996*b*) makes it impossible to use the oxidation of iron sulfides as indicative of the oxidation of other MeS (Peterson et al. 1996). Although the oxidation rate of synthesized CdS is much slower than that of FeS (Di Toro et al. 1996*b*), only one study has been conducted on the oxidation of amorphous CdS (most likely naturally occurring) in sediments (Simpson et al. 1998). The oxidation rate observed from synthesized CdS could not reproduce porewater Cd concentrations (Di Toro et al. 1996*b*). Since the oxidation of MeS releases free metal ion directly, more research is needed to quantify oxidation rates.

Resuspension of sediments as a consequence of storms or dredging exposes both the porewater and the sediment parti-

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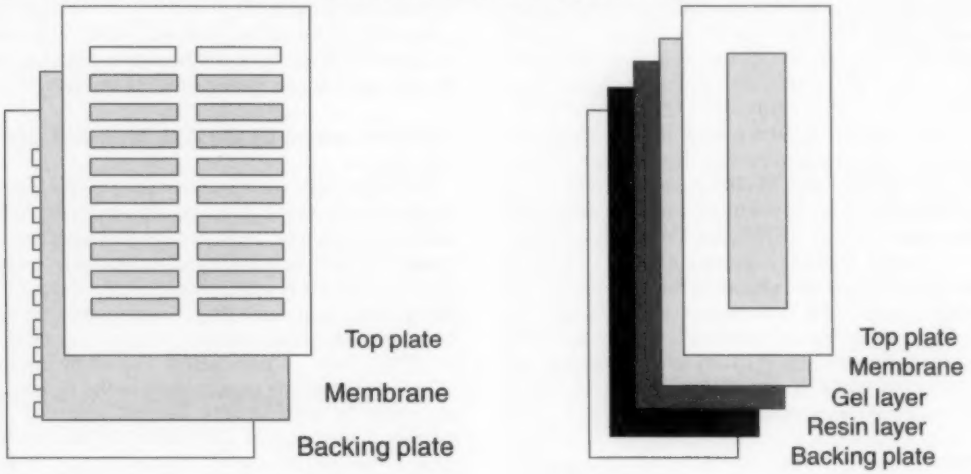
Fig. 1. Schematic view of a diffusive dialyzer (peeper) (left) and gel probe (right) for porewater sampling.



cles to the overlying water column. This can lead to an increase in the total metal and potentially the dissolved metal concentration in the water column. The oxidation of sulfide could result in the release of the associated metals. However, only a small portion of the metal contained in the sediment is released (Zhuang et al. 1994; Förstner 1995). This may be a result of scavenging of the released metals (e.g., by $Fe(OH)_3$ newly formed from the oxidation of the FeS), but is also a result of slow oxidation kinetics of many MeS. Even in a sediment that was highly contaminated by Cd, in which simultaneously extracted metals (SEM):AVS was >1, most of the Cd was not released to the water but was retained on the particles (Zhuang et al. 1994). Dissolved metal concentration following a resuspension of sediment can be less than it was prior to the resuspension (Förstner 1995).

POC in sediments is also readily oxidized either in the aerobic layer (with O_2) or in the anaerobic layer (with sulfate reduction). Since the oxidation of organic matter with sulfate as the electron acceptor is one of the sources producing AVS in sediments, a change in POC oxidation rate, which has been described by a multi-G model (Westrich and Berner 1984; Di Toro et al. 1996*b*), will also affect the concentration of AVS and hence that of soluble metals in sediment and porewaters. The multi-G model describes sulfate reduction using organic matter in sediments based on sedimentary organic matter being composed of various groups of compounds that have different reactivities with regard to decomposition (i.e., oxidation by sulfate). Each group is assumed to degrade via first-order kinetics.

Thus, in addition to chemical interactions, physical (anthropogenic and nonanthropogenic) and biological interactions can also affect the binding and release of metals from sediments. However, in general, such interactions do not appear to result in significant releases of dissolved metals.

Measurements and calculations

Porewater measurements

In general, three types of techniques have been used for porewater sampling and analysis: physical extraction from sediment cores (squeezing or centrifugation followed by filtration), in situ diffusional sampling (peepers, gel probes) (Fig. 1), and direct porewater measurement (microelectrodes). To avoid sediment oxidation and H_2S loss, all procedures must be done in a strictly non-O_2 atmosphere. Peepers, in which a membrane separates distilled water from the sediment, have been shown to provide good estimates of porewater concentrations (Carigan 1984; Carigan et al. 1985), but vertical resolution (0.5–1 cm) is limited by the size of the compartments. A new diffusional sampling technique with improved resolution (about 0.25 mm), gel probes, has been developed (Davison et al. 1991; Davison and Zhang 1994). A gel probe incorporates an ion-exchange resin separated from the solution by an ion-permeable thin-film gel, and porewater concentrations are measured by either a diffusional equilibration between the thin film of gel and the porewater (Davison et al. 1991) or a kinetic diffusion gradient in the thin film (Davison and Zhang 1994). However, the most direct technique is to use microelectrodes (potentiometric or polarographic) for which vertical resolution can reach 50–100 μm (Luther 1995). Microelectrodes are now available for pH, CO_2 (Cai and Reimers 1993), O_2 and H_2S (Revsbech et al. 1983; Visscher et al. 1991; Jeroschewski et al. 1996), and Fe and Mn (Brendel and Luther 1995) and are potentially available for other metals but are of limited use for metals at natural concentrations.

Several analytical methods are currently available for measuring metal speciation in interstitial and overlying waters, but all suffer from limitations. The most direct method to date for measuring free metal ion activities is to use metal ion selective electrodes and microelectrodes, but their application is limited by their sensitivity and selectivity (Koryta 1990). Voltammetric methods (anodic stripping voltammetry (ASV) and differential pulse anodic stripping voltammetry (DPASV)) can distinguish labile species (free metal ions, inorganic complexes, and readily dissociable weak organic complexes) from very strong organic complexes but cannot distinguish the free metal ions from other labile species (Donat and Bruland 1990; Muller and Kester 1990; Deaver and Rodgers 1996). Cathodic stripping voltammetry (CSV)

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has been extensively used to study the speciation of many of the more than 20 metals that can be analyzed in seawater (van den Berg 1998). Ligand-exchange techniques with ion-exchange resin (Mackey 1982; Sunda 1984) and ligand competition techniques with EDTA (Xue and Sigg 1994), catechol (Xue and Sigg 1993), ethylenediamine (Scarano et al. 1992), acetylacetone (Moffett et al. 1987), and ammonium pyrrolidine dithiocarbamate (APDC) (Donat and Bruland 1990) have also been used to measure metal speciation, but free metal ion concentrations are calculated from equilibrium constants rather than measured directly.

Sediment measurements

The determination of particulate metal species (metal species associated with various binding phases) in sediments is operational, dependent on the extraction procedures employed. Although sequential selective extraction methods (Tessier et al. 1979, 1989; Forstner 1982) for determining particulate metal speciation (e.g., those associated with FeOOH and MnOOH and POC) have been criticized (Kheboian and Bauer 1987; Nirel and Morel 1990), they are still pragmatic analytical tools giving insight into the geochemical processes occurring in sediments, as well as the bioavailability of trace metals in sediments (Tessier and Campbell 1991; Campbell and Tessier 1994).

AVS and its associated metals (SEM) are generally extracted with 1 M cold HCl (Allen et al. 1993) followed by sulfide analysis using gravimetric (Di Toro et al. 1990), colorimetric (Huerta-Diaz et al. 1993; Hare et al. 1994), and gas chromatographic methods (Casas and Crecelius 1994), as well as specific ion electrodes (Brouwer and Murphy 1994; Leonard et al. 1996). However, the HCl extraction will underestimate SEM values if there is more Ag in the sediment than sulfide; a 0.3 N HNO_3 extraction may be a reasonable alternative in such cases (Mahony et al. 1997). In addition, MeS such as CuS (Allen et al. 1993) and NiS (Simpson et al. 1998) are difficult to dissolve with acid during the AVS extraction. A laboratory-formulated sediment containing synthetic AVS has been synthesized (Gonzalez 1996) and may be potentially used as the quality control material for the AVS measurement.

An in situ sampling technique that can collect and separate diagenetic FeOOH, MnOOH, FeS, and MnS and AVS and their associated trace metals has also been developed (Belzile et al. 1989; Huerta-Diaz et al. 1993). The method, which involves vertically inserting a Teflon sheet in sediments in situ, has been successfully used for verifying the association of metals with naturally FeOOH and MnOOH in freshwaters (Tessier et al. 1996).

Thermodynamic calculations

Several models and computer programs have been developed or updated to calculate the distribution and speciation of metals between sediment and porewater, such as FITEQL (version 3.2; Herbelin and Westall 1996), HYDRAQL (Papelis et al. 1988), MINEQL$^+$ (version 3.01; Schecher and McAvoy 1994), MINTEQA2 (version 3.11, Allison et al. 1991), and WHAM (version 1.0; Tipping 1994). The latter is a combination of several submodels including Humic Ion-Binding Model V and is able to calculate equilibrium chemical speciation in water, sediments, and soils where chemical speciation is dominated by organic matter (humic substances).

While equilibrium thermodynamic calculations can predict metal partitioning and speciation between solid and water phases, the approach is limited by many factors: (*i*) the assumption of solid–water equilibrium is ideal whereas equilibrium may not be reached or maintained due to kinetic limitations and biological activity, (*ii*) many thermodynamic constants are given for 25°C and the results may not be valid in colder sediments, (*iii*) thermodynamic constants given in zero or low ionic strength (i.e., fresh) waters are very difficult to extrapolate to high ionic strength (i.e., marine) waters and vice versa, (*iv*) calculations are limited by the species with known formation constants (the formation constants of most metals with sulfide, polysulfides, thiols, and amino acids and the sorption (surface complexation) constants of metals on naturally occurring FeOOH and MnOOH and organic matter are not well known), and (*v*) the exact crystalline forms of MeS pertinent to natural waters and their solubility products are still not well understood. However, despite their limitations, thermodynamic calculations are being used with increasing frequency and are useful for process elucidation.

Bioavailability pathways

Bioavailable species: free metal ion versus other species

There are two models that can describe transport of metals into a cell. The first assumes a diffusion layer in which dissociation and exchange of metal complexes occur. The kinetics of this process are limiting and transport into the cell is rapid. The second, the free-ion activity model (FIAM), described below, assumes that metal transport from solution to the cell membrane and the subsequent surface complexation reaction occur rapidly, such that an equilibrium is reached at the cell surface and the limiting factor is transport into the cell.

A number of studies (as reviewed by Campbell 1995) have implied that the free aquo ion (Me^{2+} or $Me(H_2O)_x^{2+}$) is the most bioavailable species of metal. The effect of water chemistry (such as pH, alkalinity, ligands, E_h) on metal bioavailability can be considered as its influence on the free metal activity in solution and (or) ion competition with binding sites on the cell, gill, or gut surface for the free metal ion. To rationalize these experimental observations, the FIAM has been proposed (Morel and Hering 1993; Campbell 1995). The model assumes that the interaction between metal and organism can be described as a surface complexation reaction occurring at the surface of the plasma membrane:

$$(14) \quad \equiv\text{Cell} + \text{Me}^2 = \equiv\text{Cell-Me}, K_{\equiv\text{Cell-Me}}$$

$$(15) \quad [\equiv\text{Cell-Me}] = K_{\equiv\text{Cell-Me}}[\equiv\text{Cell}]\{\text{Me}^{2+}\}.$$

If the biological response is dependent only on the concentration of the formed surface complex [≡Cell-Me], and the concentration of reactive sites at the cell surface [≡Cell] is constant, the biological response is then proportional to the free-ion activity in the solution. The critical review by Campbell (1995) supports the FIAM model, particularly in cases without natural dissolved organic matter (DOM) where

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the FIAM may not be accurate (e.g., Roy and Campbell 1997). Field studies on bioaccumulation of Cd by a freshwater bivalve (*Anodonta grandis*) (Tessier et al. 1993) and a freshwater insect lava (*Chaoborus punctipennis*) (Hare and Tessier 1996) in Canadian lakes also support its use in predicting metal concentrations in aquatic organisms in nature.

However, examples of where metal toxicity seemingly is not well correlated with free metal ion activity (Campbell 1995; Erickson et al. 1996) suggest that other labile metal species (hydroxyl complexes (Cowan et al. 1986; Fisher 1986), organometallic species (e.g., butyltin, methyl mercury), oxyanions (e.g., chromate, arsenate), and possibly humic substances (Stackhouse and Benson 1989)) and nonchemical variables (Erickson et al. 1996) are also important in determining metal bioavailability. In addition, the chemistry of the biotic receptor site can be modified by changes in the solution chemistry, particularly by changes in pH and hardness. Further, carefully defined experiments on FIAM with organic ligands are particularly needed (Campbell 1995).

A mechanistic approach to understanding the influence of solution-phase complexation on the toxicity of metals was proposed by Pagenkopf (1983). He treated metal ion receptor sites on the gills of fish as chemical entities that compete with inorganic and organic ligands in the solution for metal ions. This allows prediction of the interaction of the metal with the organism and prediction of the competitive effects of Ca^{2+}, Na^{+}, and H^{+} based on equilibrium considerations. Models such as the WHAM model (Tipping 1994) can be extended to incorporate biotic receptor sites as well as the nonbiotic ligands.

The principal receptor site for toxic metals in freshwater fish is the gill where Na^{+} and Ca^{2+} are transported from the bulk water to the bloodstream by active, energy-requiring "pumps" (Wood 1992). The channel or carrier proteins associated with these pumps occur as specific, negatively charged ligands on the gill surface. Thus, the gills of freshwater fish have the important physiological functions of gas transport (O_2, CO_2, NH_3), active uptake of ions (Na^{+}, Ca^{2+}), and elimination of ions (Cl^{-}). Playle et al. (1992, 1993*a*, 1993*b*), MacRae (1994), and Janes and Playle (1995) have treated the specific receptor sites on the gill as competitive ligands for the binding of trace metals. They have determined conditional stability constants and site densities for these surface complexation reactions through competitive binding experiments with ligands whose complexation constants are known. MacRae (1994) showed that there is a strong relationship between the extent of saturation of the gill receptor sites and mortality. Experiments by these research groups have shown that gills compete for metals with natural DOM.

An important conclusion from the gill complexation model is that bioavailability of metal species is at least partly a function of biological mechanisms. The presence of the gill causes chemical reequilibration in the system. It is the degree to which the gill complexation sites are occupied by specific metal species that determines whether toxicity will occur. A similar biotic site binding model incorporating both metal speciation in sediment and porewater and the interaction of the metal with benthic organisms is necessary to predict toxicity for systems of differing chemistry. Such a model would greatly improve our ability to model and predict speciation and toxicity.

Relative importance of sediment versus interstitial water versus overlying water versus food

Although total metal concentrations in sediments do not correlate with sediment biological effects, the correlation for some metals is improved when sediment metal concentrations are normalized with respect to AVS (Di Toro et al. 1990, 1992; Ankley et al. 1996) or AVS and POC (Mahony et al. 1996; Ankley et al. 1996) in anaerobic sediments and iron hydroxide and (or) POC in aerobic sediments (Tessier and Campbell 1987; Campbell and Tessier 1994). The dependence of trace metal bioavailability on sediment characteristics (binding phases) does not imply necessarily that the main pathway of metal bioavailability is via ingestion of particulate metals in sediments (Tessier and Campbell 1987). Furthermore, particulate-associated metals seem not to be directly bioavailable (Campbell and Tessier 1994; Deaver and Rodgers 1996). As previously noted, it is most likely that the sediment metal concentration and sediment–water chemistry (metal-binding phases in sediments, pH, E_h, ligands, etc.) control free metal ion activity in porewater, while free metal ion in porewater directly causes biological effects. For example, toxicity of Cd in marine sediments to amphipods can be accurately predicted from concentrations in porewater (Swartz et al. 1985; Kemp and Swartz 1986).

Strong relationships between free metal concentrations (e.g., Cd, Cu) in overlying waters and metal bioavailability to benthic organisms have also been reported, either from laboratory experiments (O'Donnel et al. 1985; Deaver and Rodgers 1996) or from field investigations (Tessier et al. 1993; Hare and Tessier 1996). This is generally observed for tube-dwellers (e.g., oligochaetes, polychaetes, larvae of several genera of Diptera) and large benthic animals (e.g., crustaceans, molluscs), probably because their bioirrigation and bioturbation of surficial sediments afford them more exposure to overlying water (Deaver and Rodgers 1996). However, even in these cases, metal bioavailability (expressed as either metal concentration in organisms or free metal ion concentration in overlying waters) is well-correlated with sediment metal concentration normalized by appropriate metal-binding phases (e.g., POC and FeOOH as observed by Tessier et al. (1993) and POC and AVS as reported by Mahony et al. (1996)).

The general relationship between bioavailability and free metal ion activity in water does not mean that the main pathway of metal bioavailability is directly from water in all cases. For example, Munger and Hare (1997) found that there was no significant difference between the Cd concentrations in *C. punctipennis* exposed to the metal in its food (algae and crustaceans) alone versus those exposed to Cd in both food and water, indicating that the animal took the majority of its Cd from food, while Cd bioaccumulation from water was negligible. A benthic herbivore, the freshwater crustacean *Hyalella azteca*, was also reported to accumulate the bulk of its Cd from food (Stephenson and Turner 1993). However, Fisher et al. (1996) found that water was a more effective metal source to the mussel *Mytilus galloprovincialis* than food. Metals obtained from food were primarily bound to soft tissues, while metals from water were

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mostly associated with the mussel shells. Gut uptake of metals may be an extremely important source of exposure in some cases, but definitive work on this subject remains to be done.

Metal bioavailability prediction

General considerations

Modelling bioavailability of metals in sediments and any subsequent biological effects requires consideration of both the possible sources of the metals (interstitial waters, overlying waters, food) and whether the site of response is external (e.g., gills) or internal. If the effect is internal, according to FIAM and assuming that the three possible metal sources to organisms are additive, metal bioavailability can be expressed as

$$(16) \quad Me_{Bio} = f_1\{Me^{2+}\}_{IW} + f_2\{Me^{2+}\}_{OW} + f_3[Me]_{Food}$$

where $\{Me^{2+}\}_{IW}$ and $\{Me^{2+}\}_{OW}$ are the free metal ion activities in interstitial and overlying waters, respectively. The concentration of metal in food, $[Me]_{Food}$, is proportional to the concentration of the free metal ion in the digestive tract. The fraction of the total metal attributable to each of the three sources is given by the appropriate value of f.

Hypothesizing that the metal in food is taken from porewater and (or) overlying water, and the food organisms are in equilibrium with their environment, then:

$$(17) \quad [Me]_{Food} = f_4\{Me^{2+}\}_{IW} + f_5\{Me^{2+}\}_{OW}.$$

The free metal ion activity in both interstitial water and overlying water can be predicted from the sediment metal concentration normalized by certain factors, as previously discussed:

$$(18) \quad \{Me^{2+}\}_{IW} = f_6[Me]_{sed,norm}$$

$$(19) \quad \{Me^{2+}\}_{OW} = f_7[Me]_{sed,norm}.$$

The overall bioavailability is then a function of the normalized sediment metal concentration:

$$(20) \quad Me_{Bio} = f[Me]_{sed,norm}.$$

On the other hand, if the biological effect is external, that is at a membrane rather than intracellular, then the bioavailability can be expressed as

$$(21) \quad Me_{Bio} = f_8\{Me^{2+}\}_{IW} + f_9\{Me^{2+}\}_{OW}.$$

This simple relationship indicates that, even though metal pathways to biological effects are not clear (i.e., versus water, sediment, or food), metal bioavailability in sediments can probably be predicted by either the free metal ion concentration in waters (interstitial water and (or) overlying) or metal concentrations in sediments after certain normalization(s).

Interstitial and overlying water approaches

Although free metal ion activity in interstitial or overlying waters is probably the most direct and reasonable predictor for metal bioavailability, its application is limited by a lack of efficient analytical techniques for measuring free-ion activity, as previously discussed. To avoid difficulties in measuring free metal ion activity, the total dissolved metal concentration normalized by water-only exposure toxicity measurements, interstitial water toxicity unit (IWTU) (Berry et al. 1996; Hansen et al. 1996*a*) and interstitial water criteria toxicity unit (IWCTU) (Ankley et al. 1996), has been proposed for predicting the metal-contaminated sediment toxicity:

$$IWTU = \sum([Me]_{i,IW}/LC_{50,i})$$

$$IWCTU = \sum([Me]_{i,IW}/WQC_i)$$

where $[Me]_{i,IW}$ is the soluble concentration of metal i in interstitial water, $LC_{50,i}$ is metal i's water-only exposure LC_{50} for the test organism, and WQC_i is metal i's water quality criteria (final chronic values, FCV). It was generally found that no significant toxicity occurs to test organisms in sediments when IWTU < 0.5 (Berry et al. 1996; Hansen et al. 1996*a*). The measurement of IWCTU has recently been proposed as one of the approaches for deriving sediment quality criteria for metals (Ankley et al. 1996). However, it should be noted that LC_{50}s and other water-only exposure toxicity endpoints are normally given as total concentrations and may vary with water chemistry and the test organisms. In general, because of the more extensive complexation of metal in porewater than in other waters, the LC_{50} would be expected to be greater for porewater exposure. In addition, the relationship between bioaccumulation and IWTU or IWCTU has seldom been reported.

Sediment approaches

Since bioavailable metal from interstitial water, overlying water, or food is likely controlled by metal concentration in sediments and sediment characteristics, and metal concentration in sediments can be measured more conveniently, metal bioavailability prediction based on (normalized) metal measurements in sediments is a feasible approach to regulation.

Based on the relative importance of metal-binding phases in sediments (discussed previously), several normalization factors have been proposed. In anaerobic sediments where AVS is the predominant binding phase of metals, free metal ion activity is controlled by the dissolution of MeS. Since the $K_{sp,MeS}$ of all the metals other than Fe and Mn are very low, if there is no other source of free metal ion, the activity of free metal ion in interstitial waters, $\{Me^{2+}\}$, will be too low to cause toxicity. Based on this relation, Di Toro et al. (1992) have proposed an AVS/SEM model, which predicts that no sediment toxicity would be observed when the molar concentration of AVS is greater than the SEM ([SEM]/[AVS] < 1), while toxicity may or may not occur when [SEM]/[AVS] > 1. The model has been verified for five divalent metals, including Cd, Cu, Ni, Pb, and Zn, by short-term (Di Toro et al. 1990; Carlson et al. 1991; Casas and Crecelius 1994; Pesch et al. 1995; Berry et al. 1996; Ankley et al. 1991, 1993; Hansen et al. 1996*a*) life cycle (De Witt et al. 1996; Sibley et al. 1996) and long-term (Hansen et al. 1996*b*) laboratory and field (Hare et al. 1994; Liber et al. 1996) toxicity experiments. The model has also been proposed for deriving sediment quality criteria for metals (Ankley et al. 1996). However, caution is advised when using the AVS/SEM model for assessing metal bioavailability in sediments, since even in the case of AVS > SEM, (*i*) metal ion activity can be high enough to cause toxicity

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when pH is very low (eq. 4), (*ii*) temporal and spatial variation and oxidation of AVS and SEM can increase metal bioavailability significantly, and (*iii*) the SEM/AVS model is based on the relationship between toxicity and free metal ions. While the free metal ion dominates expression of toxicity, it is not the only bioavailable form of the metal; toxicity may be observed when a large quantity of ligands is present in porewaters. Ligands reduce the availability of metals in most circumstances through complexation of metals and reduction of the free-ion activity. In some porewater situations, they may increase metal availability by increasing the amount of soluble metal that can diffuse to biotic surfaces. The application of the AVS/SEM model is also complicated by variation of the AVS/SEM ratio in the vertical sediment profile. SEM may show a flat profile (no change with depth) or be elevated near the surface (recent contamination) whereas the AVS profile is usually elevated in deep sediments. The AVS/SEM ratio may hence be $<<1$ in surface sediments while $>>1$ in deep sediments (van den Berg et al. 1998)

Some MeS (e.g., CuS, Allen et al. 1993; NiS, Simpson et al. 1998) do not dissolve under the current method for measuring AVS/SEM. This does not invalidate the AVS/SEM methodology to assess bioavailability. If the MeS does not dissociate in the acid, both the numerator and the denominator of the inequality will be reduced by the same molar amount relative to their amounts if the MeS did dissociate. Thus, nondissociation of an MeS will not cause an AVS/SEM < 1 to become >1, nor will an AVS/SEM > 1 become <1.

In sediments where little or no AVS is present, particularly in aerobic sediments, sediment metal concentration normalized by POC and (or) FeOOH has been shown to be predictive of biological effects. Normalization factors include extracted Fe with $NH_2OH{\cdot}HCl$ (Tessier et al. 1984), HCl (Bryan 1985), in situ collected diagenetic Fe (Tessier et al. 1993), and POC (Tessier et al. 1993). Notably, Tessier et al. (1993) found that the free Cd^{2+} concentrations in the overlying waters of 38 Canadian lakes are better predicted from the POC- and FeOOH-normalized sediment Cd concentrations than from the total dissolved Cd concentrations in overlying waters. However, normalization by POC, FeOOH, and MnOOH depends on the availability of corresponding binding constants, which are still not well understood.

Tools for hazard identification and ERA

Bioavailability

The key to correctly identifying the hazard and risk posed by metals in sediments is evaluation of their exposure potential which includes bioavailability. Even relatively high levels of contamination may be of little or no biological significance if bioavailability is limited (Power and Chapman 1992; Allen 1996). Considerable progress has been made in the development of predictive tools for assessing metal bioavailability (Ankley et al. 1996). However, such tools (e.g., sediment quality values) are only useful for screening assessments, not for final decision-making (Chapman 1997*b*). The only reliable way to actually measure, proactively, the bioavailability of metals in sediments is to perform bioassays (i.e., toxicity including investigative tools such as sediment toxicity identification evaluations (TIEs) and (or) bioaccumulation tests). Such proactivity can involve "worst-case" testing for existing or possible situations. Bioavailability can also be assessed by correlative means but, reactively, by assessing community structure relative to contamination.

Sediment quality can be assessed by a variety of laboratory and field methods in the following general categories (adapted from Chapman et al. 1992), discussed below: sediment chemistry, toxicity assessments including biomarkers, bioaccumulation measurements, and community structure analysis. The overall goal of ERAs performed on metal contaminated sediments is to evaluate potential effects on population/community structure, function, and composition.

Sediment chemistry

Historically, evaluating the risk of metal-contaminated sediments has involved identification and quantification of the contaminants (exposure assessment) followed by a comparison with numeric criteria (i.e., sediment quality values). This traditional sediment quality assessment approach is often based on concentration – adverse effect relationships established in laboratory tests for individual metals and on single (test) species compared with measurements of metals in bulk sediments. Although chemical analytical measurements are necessary to investigate the composition and magnitude of sediment contamination and to identify possible contaminant sources, these determinations, alone, are inadequate tools for fully characterizing and (or) predicting potential biological effects (Ginn and Pastorok 1992); metals derived from the "aqueous" phase of contaminated sediments are thought to be the primary source of metal toxicity.

Sediment toxicity

Risk predictions made by comparison of sediment metal measurements (exposure) with biological effects estimates require confirmation by either in situ or laboratory biological effects measurements.

Laboratory tests: sediment handling

Laboratory studies require collection of sediment, storage, and, depending on the method, extraction of the relevant test fraction. All these steps can have relatively drastic effects on sediment integrity, disrupting physicochemical and biological gradients and their relationships, which in turn may affect toxicant partitioning, complexation, speciation, and, ultimately, bioavailability (e.g., Malueg et al. 1986; Stemmer et al. 1990; Mudroch and MacKnight 1991; Burton 1992*a*). Cu, Fe, and Zn appear to be particularly sensitive to sample handling (Mudroch and Bourbonniere 1991).

Burton (1992*a*) reviewed how various aspects of sediment collection and processing can affect bioavailability. Drying, freezing, and storage temperature have all been shown to affect the toxicity of stored metal-contaminated sediments (ASTM 1994). Recommended storage time limits for metal-spiked sediments vary from 2 (Stemmer et al. 1990) to 7 days (Anderson et al. 1987) to less than 2 weeks (ASTM 1994). Exposure to air must be minimized, as this might re-

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duce or increase toxicity due to oxidation and precipitation of metal species or loss of AVS. Field-collected sediments should be stored at 4°C with no airspace or under nitrogen and should be tested as soon as possible after collection (Becker and Ginn 1995) and at least within 8 weeks (U.S. EPA/ACOE 1994).

In addition to testing whole sediments, sediment toxicity is also assessed by evaluating the toxicity of porewater or of elutriate. While all toxicity tests are carried out on oxygenated porewaters irrespective of their initial redox status, collection of porewater or the preparation of elutriates may further alter sediment toxicity (e.g., by affecting the redox stability of the samples, thus affecting metals in porewaters). The different methods of collecting sediment interstitial water (centrifugation, squeezing, suction, equilibrium dialysis) can all produce experimental artifacts (Malcolm et al. 1990; Adams 1991; Bufflap and Allen 1995). For example, while centrifugation followed by 0.2-μm filtration did not change the (chemical) porewater composition of metal-contaminated sediments (Carigan et al. 1985), the latter step significantly changed the toxicity of porewater containing nonpolar organics (Burton 1992*a*). Storage of porewaters can also alter their chemistry and result in the formation of precipitates. Storage-induced changes in marine porewater composition have been reported by Hulbert and Brindle (1975), while Landrum et al. (1987) observed coagulation and precipitation of the humic material in porewaters stored for more than 1 week. However, porewater is an important test medium. Ankley et al. (1994) concluded that, for a variety of benthic invertebrates, porewater concentrations of metals correspond well to the bioavailability (toxicity) of metals in test sediments.

An integrated quantitative assessment of the relative importance of the various steps involved in porewater toxicity testing of metal-contaminated sediments was undertaken by Janssen et al. (1995) who examined, in a multifactorial test design, the influence of sample collection, storage, and porewater extraction method on the toxicity of a sediment contaminated with various organics and metals. Sampling procedure (grab versus core) in combination with storage conditions (O_2 versus N_2 atmosphere) proved to be the most important factors affecting the toxicity of the extracted porewaters. In general, grab samples stored under non-N_2 conditions resulted in the highest toxicity. Extraction of the porewaters by centrifugation (versus squeezing) resulted in higher toxicity. The effects of other extraction variables such as O_2/N_2 atmosphere, temperature, and filtration proved to be of minor importance.

Formulated sediments have been proposed for use as reference sediments (Walsh et al. 1990, 1992; Suedel and Rodgers 1994*a*, 1994*b*) and for spiking experiments (Burgess et al. 1994; Harrahy and Clements 1997). Such formulations, although artificial, do address some of the above issues related to field-collected sediments and also allow for the possibility of more closely matching diverse sediment types than is normally possible by field sampling. For instance, a formulated reference sediment incorporating synthetic AVS has been proposed (Gonzalez 1996). Similarly, formulated dilution waters have been advocated for use in sediment toxicity testing (Smith et al. 1997).

Laboratory tests: toxicity testing

Benthic test species used in whole-sediment exposures are the best indicators of sediment toxicity due to their direct contact with sediments and interstitial waters and the proven effectiveness of such species in detecting sediment toxicity (Burton et al. 1992). Amphipods are commonly used in fresh, estuarine, and marine waters for assessing sediment toxicity (Chapman 1997*a*), along with a variety of other species (Lamberson et al. 1992).

Nonbenthic aquatic species (which do not reside in the sediment for an extended portion of their life cycle) have been used in hazard assessments of contaminated sediments since the late 1970s and include bacteria, phytoplankton, macrophytes, zooplankton, fish, and amphibians (Burton 1992*b*). Di Toro et al. (1991), on the basis of an extensive comparative literature review, concluded that when both infaunal and epibenthic species are classified as benthic, on average, they have a similar sensitivity to water column species.

Sediment testing with water column species has generally involved elutriates, porewaters, or overlying waters (Ross et al. 1988; Munawar et al. 1989). Some water column species may be more relevant than others. For instance, the freshwater planktonic cladocerans *Daphnia* sp. and *Ceriodaphnia dubia* that have been used in whole-sediment, suspended-sediment, and slurry tests (Burton 1992*b*) spend a considerable amount of time feeding on the sediment surface and have been found to have a comparable sensitivity to benthic species, both in the laboratory and in situ (Giesy et al. 1988, 1990). Fathead minnow (*Pimephales promelas*) has been the species of choice in freshwater sediment toxicity tests (Burton 1992*b*). The fathead minnow 7-day early life stage test, originally developed for effluent testing, has been adapted for use with sediment extracts (Dawson et al. 1988), elutriates (Hoke et al. 1990), interstitial waters (Ankley et al. 1990), and whole sediments (U.S. EPA 1990). Although tests with aqueous extracts such as porewaters or elutriates do not, for organic contaminants, accurately predict contaminant bioavailability compared with using whole sediments (Harkey et al. 1994), this may not be true for metals that can generally be extracted more efficiently from sediments than organic compounds (Carigan et al. 1985). Ingersoll et al. (1995) reviewed freshwater sediment toxicity (and bioaccumulation) testing and recommended integration of different methods via weight of evidence.

In situ tests

In situ assays of contaminated sediments have become a powerful tool for both the validation of laboratory results and the assessment of potential (site-specific) sediment toxicity. Although various in situ test designs that include sediments have been developed (e.g., enclosures, artificial streams and ponds (Cairns 1985), caging experiments with various species (Sasson-Brickson and Burton 1991)), few of these studies have focused directly on sediment toxicity. A more direct way to examine the effects of contaminated sediments is the use of in situ test chambers (basically test containers allowing relatively free exposure to the ambient medium: Sasson-Brickson and Burton 1991; Chappie and Burton 1997) that remove sampling- and laboratory-induced

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error in the assessment process. Greater toxicity generally occurs in the laboratory (confirming that laboratory tests are "worst-case" studies) than in situ (chambers placed in the field) for metals and other substances (Sasson-Brickson and Burton 1991; Skalski 1991). Although in situ studies eliminate sampling and manipulation effects, in many cases, they are impractical if not impossible to perform.

Biomarkers

Biomarkers are biochemical, physiological, or pathological responses measured in individual organisms that provide information concerning exposures to environmental contaminants and (or) sublethal effects arising from such exposures (Benson and Di Giulio 1992). Despite considerable interest in the development and application of biomarkers, the interpretation of most biomarker signals in an ecotoxicological context (e.g., the relationship between biomarker level effects and population level effects) is, at best, very difficult (Huggett et al. 1992). The potential application of these techniques to hazard identification and risk assessment including contaminated sediments is reviewed in Benson and Di Giulio (1992), HERA (1996*a*), and Decaprio (1997).

Few studies have been conducted using biomarker techniques to evaluate the hazard of metal-contaminated sediments. Metallothioneins (MTs) have the best potential as candidate biomarkers for this purpose. Although the precise function of MTs (metal-binding proteins in animals, plants, and prokaryotes) is still unclear, they seem to play a major role in essential metal homeostasis. Perhaps more important from an applied point of view, MTs are also induced at relatively low exposures to trace metal contaminants. Beyer et al. (1994) reported on the measurement of MTs as part of a battery of biomarkers in caged European flounder (*Platichthys flesus*) to evaluate contaminated sediments near metal smelter industries. Pedersen et al. (1997) examined MT concentrations and stress protein levels (HsP70) in shore crab (*Carcinus maenas*) collected from metal-contaminated sediments. Both Cu and Zn MT concentrations in the gill tissue reflected the trends in metal contamination in the underlying sediments as well as the exposure gradients. In contrast, HsP70 levels in the gills did not appear to correlate with the degree of exposure to trace metals. Hylland et al. (1992) assessed the effect of metals on dab (*Limanda limanda*) in the German Bight using hepatic levels of MTs and metal tissue levels. Hepatic MTs and metal levels did not reflect metal concentrations in the sediment, but were consistent with elevated metal content of two invertebrate species.

Other biomarkers may also be useful in future. For instance, Hamoutene et al. (1996) measured thiobarbituric acid reactive species (TBARS) content in Cd-exposed seagrass (*Posidonia oceanica*) as an indicator of oxidative stress (lipid peroxidation). Biomarkers such as immunological responses and physiological indices (glycogen and lipid content and blood chemistry indicators) have only rarely been used to assess the hazard of contaminated sediments, especially metal-contaminated ones (Benson and Di Giulio 1992). Meinelt et al. (1997) examined the effect of Hg-contaminated sediment on the presence of macrophage centers (MC) in various tissues of northern pike (*Esox lucius*) and found a positive correlation between Hg concentration and the MC response.

In a novel approach, Andreasson and Dave (1995) examined the transfer of heavy metals from sediment to the salmonid *Oncorhynchus mykiss*. Uptake and biliary excretion of metals were studied in fish exposed to a mixture of seven heavy metals. Metal concentrations and toxicity (*Daphnia magna*) of the bile were used as indicators of exposure. The authors concluded that the potential use of bile toxicity as a biomarker of heavy metal exposure in fish is probably limited by the low bioconcentration of many of the toxicants in bile.

Bioaccumulation

Bioaccumulation measurements indicate bioavailability but are not yet reliable predictors of bioeffects, and thus are inappropriate for hazard assessment of metals (Chapman et al. 1996*a*, 1996*b*); they are more appropriate for risk assessment of metals in sediments (Ankley 1996; Chapman 1996*a*; Harrahy and Clements 1997) particularly related to possible secondary poisoning (accumulation of metals through food over a single trophic level), possibly resulting in adverse effects on the predator (Woodward et al. 1993; Munger and Hare 1997). The use of bioaccumulation measurements to predict adverse impacts is in its infancy, but shows promise (Chapman 1997*c*; Borgmann and Norwood 1997*a*, 1997*b*).

Sediment contaminants, if bioavailable, can be bioaccumulated by aquatic organisms, possibly giving rise to acute or chronic health effects (Lee 1992). Inorganic metals are not biomagnified (i.e., accumulated through food over two or more trophic levels, Chapman et al. 1996*a*, 1996*b*). A direct approach to monitor or predict bioaccumulation is the quantification of contaminant tissue residues in field-collected or laboratory-exposed organisms. A less costly but also less precise alternative is the prediction of tissue concentrations using sediment bioaccumulation models: equilibrium-based approaches that assume steady-state conditions between the organism and the environment or kinetic approaches that describe bioaccumulation as the net effect of rate processes (Lee 1992). However, the development of such predictive tools has to a large extent concentrated on the bioaccumulation of neutral organic compounds (Lee 1992; Mac and Smith 1992).

Although a majority of studies on metal bioavailability in sediments focus on either acute or chronic toxicity, bioaccumulation may be a more accurate endpoint for assessing bioavailability in risk assessments (Ankley 1996). The metal (expressed either as total concentration or as a specific species) may be bioavailable even if toxicity is not observed, since toxicity is related to the test animal's sensitivity and the length of the test. While toxicity experiments are often done in the laboratory, measurements of bioaccumulation can be more feasible for field investigations.

However, it is important to note that organisms actively maintain internal concentrations of essential metals (e.g., Cu, Zn) through the use of homeostatic mechanisms. In these cases, there is no relationship between porewater or overlying water exposure and a bioconcentration factor (BCF, Chapman et al. 1996*a*).

Field studies

The ecologically most relevant way to assess the risk of metal-contaminated sediments to aquatic biota is the "holis-

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tic" approach of conducting in situ assessments of the degree of disturbance of exposed biological communities. However, this approach is correlative, not predictive, does not establish cause and effects links, and is not effective in discriminating the degree of contamination by metals (Diaz 1992; La Point and Fairchild 1992). In cases of moderate contamination, it is very difficult, if not impossible, to discriminate effects due to natural factors from those resulting from the impact of metals or other contaminants. This is even more difficult for contamination resulting from diffuse sources and in areas with high natural variability (Luoma 1995) than in, for instance, rivers subject to specific mining/smelting activities (McGuire 1989).

Aside from the structure of benthic biological communities, the impact of metals in contaminated sediments has also been studied in situ through measurement of particular functional endpoints related to energy flow and material cycling, such as production and respiration rates. For example, Mills and Colwell (1977) and Burton et al. (1987) studied the enzymatic activity of naturally derived sediment microbes exposed to metals in a metal-impacted stream. Medine et al. (1980) and Scanferlato and Cairns (1990) followed O_2 production and consumption rates in naturally derived sediment–water microcosms spiked with metals.

Cairns et al. (1992), in their review of functional attributes as endpoints in ecotoxicology, concluded that functional measures are not a common component of field studies. However, in contrast, Anderson et al. (1987) suggested that microbial community O_2 demand is the sole functional measure that needs to be included in any list of endpoints for assessment of contaminated sediments.

Sediment metal toxicity has also been assessed in situ through biological monitoring of populations of particular species, mainly macroinvertebrates (Elliott and McLusky 1985; Luoma 1995). The "indicator species" concept lacks broad applicability to all types of contaminated sediments (Diaz 1992) but, at least in freshwaters, particular taxa (e.g., Ephemeroptera, Plecoptera, Trichoptera) appear to be sensitive to metals and other inorganic contaminants, and hence may provide a useful reactive index for initial screening.

Integrated approaches

The various assessment tools described above differ greatly in their ability and suitability to assess the hazards and risks of metals-contaminated sediments. It is therefore important to match assessment methods to the site- and program-specific objectives of the study being conducted (U.S. EPA 1992). Unfortunately, there is no single method that will measure all contaminated sediment impacts at all times and on all biological organisms. Thus, a "weight of evidence" approach that integrates several types of methodologies is often the most useful. In this regard the sediment quality triad (Triad) approach integrates the results of bulk sediment toxicity testing, benthic community structure analysis, and chemical sediment analysis (Chapman et al. 1992; Chapman 1996*b*; Suter 1996). Pascoe (1993) applied the Triad approach to performing risk assessments of metal-contaminated wetlands. Canfield et al. (1996) used the Triad to determine the nature and extent of (metal) sediment contamination in the Great Lakes Areas of Concern. The authors concluded that laboratory sediment toxicity tests may better identify chemical contamination in sediments than many commonly used measures of benthic invertebrate community composition, which are also affected by other factors such as habitat alterations. Other examples of the Triad applied to metals-contaminated sediments include Canfield et al. (1994), Pascoe et al. (1994), and Besser et al. (1996*b*).

Integrated approaches such as the Triad are more appropriate for risk assessment than for hazard assessment. Measuring sediment chemistry and comparing the results with sediment quality values provides the simplest approach to risk identification. Sediment toxicity tests (in the laboratory or, better, in situ) appear to provide the best single approach to hazard assessment that actually accounts for bioavailability. These approaches, used independently or in combination (Wildhaber and Schmitt 1996), can subsequently be cost-effectively integrated through a tiered approach (Chapman 1996*b*) into a full ERA.

ERA

The process

Risk assessment of sediments (and other media) consists of four major steps in both Europe and Canada (Chapman 1996*a*; Fleming et al. 1997): hazard identification, dose–response (or effects) assessment, exposure assessment, and risk characterization. The U.S. EPA (1992) three-step procedure for risk assessment involves problem formulation, analysis (exposure and effects characterization), and risk characterization. The term "risk assessment" is used here in reference to these specific, formalized procedures.

Hazard identification (also called hazard assessment or hazard characterization) refers to the identification of potential adverse effects. ERA refers to the integration of exposure and effects data to judge the potential for effects to occur or to have occurred. Effectively, identifying hazard means identifying a source of danger; determining the potential that this danger will result in actual harm to the environment requires ERA.

Whether potential hazard due to the presence of metals in sediments may be realized depends on the magnitude of the exposure and the bioavailability of the metals in sediments. The dose–response assessment provides information on the relationship between sediment metals concentrations and the incidence and (or) severity of adverse effect(s). Exposure assessment determines the emissions, pathways, and rates of movement of metals into and out of sediments, including transformations, in order to estimate the concentrations to which biological systems are or may be exposed. The final step, risk characterization, integrates the previous three steps to estimate the incidence and severity of any adverse effects likely to occur. Risk characterization also includes an uncertainty assessment of each component and of the overall risk assessment.

Risk assessments consider two time scales: short term (i.e., acute) and long term (i.e., chronic). They also consider two spatial scales encompassing both narrow and wide ranges of environmental conditions: local and regional. Risk assessments of substances such as metals typically involve quotient approaches, specifically comparing their predicted environmental concentration (PEC, determined from the exposure assessment) with their predicted no effect concen-

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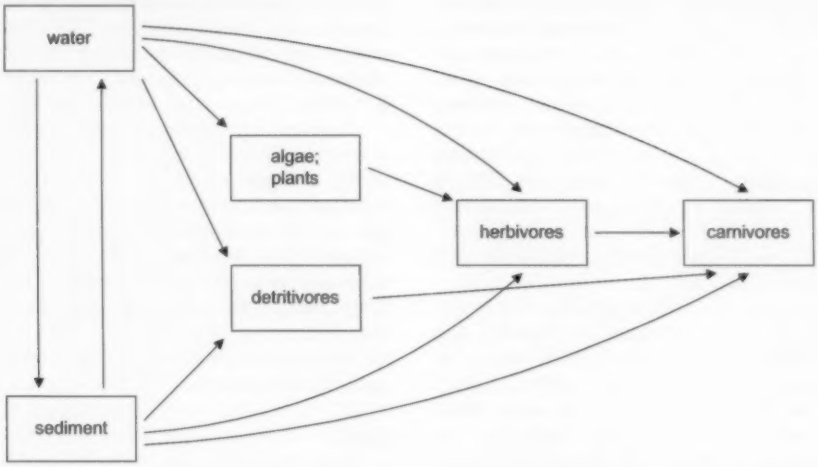
Fig. 2. Metal pathways to benthic organisms.



tration (PNEC, determined from the dose–response assessment). PECs can be measured; PNECs cannot be measured directly but are inferred based on toxicity studies and best professional judgement. PNECs are often derived by applying a safety factor to the lowest measured no observed effect concentration (NOEC) or a statistical extrapolation. The latter is preferable to the former; single point estimates are not as useful as probabilistic distributions (Chapman et al. 1998).

The overall intent of any ERA is to evaluate risk to populations and communities in the field. Thus, data from well-defined field studies, for both exposure and effects, are preferable (U.S. EPA 1997).

ERA of metals in sediments

Ingersoll et al. (1997) discussed three specific applications of contaminated sediment ecological risk assessment (SERA): navigational dredging, contaminated site cleanup, and product safety assessment. The first two applications are retrospective (conducted after sediments are contaminated); the third application is prospective or predictive (conducted before sediments are contaminated). Ingersoll et al. (1997) recommend the use of weight of evidence, or the generation of multiple lines of evidence, to support decision-making such that no single line of evidence, alone, drives decision-making (Suter 1996). Weight of evidence can be defined as (Menzie et al. 1996) "...the process by which measurement endpoints are related to an assessment endpoint to evaluate whether a significant risk of harm [an unacceptable risk outcome] is posed to the environment." Assessment endpoints are explicit expressions of an actual environmental value to be protected (e.g., maintenance of resident communities); measurement endpoints are the lines of evidence that evaluate the assessment endpoints (e.g., survival, growth, or reproduction in the laboratory, species diversity measurements).

Risk assessment of metals in sediments requires specific considerations within the accepted, generic approach (a new paradigm is not required). These considerations are outlined below in terms of both general issues and issues specific to each of the four stages of a risk assessment, focusing on retrospective assessment. Generic risk assessment components that are not specific to metals or noteworthy are not discussed below; details are provided in Ingersoll et al. (1997). Tools for ERA (and hazard identification) of metals-contaminated sediments have been discussed. Pathways of metals to organisms are shown in Fig. 2.

General considerations

Risk assessment for metals in sediments must take into account three separate possible transformation processes that can occur simultaneously but not necessarily sequentially (Chapman 1996*a*): dissolution (solubilization), transformation to bioavailable forms, and transformation/sorption to nonbioavailable forms. When a metal enters the aquatic environment, solubilization can occur, which may increase bioavailability. However, the ultimate fate of metals in water is association with sediments, which will make them less bioavailable (i.e., persistence reduces risk; the reverse is the case for organic chemicals). Risk assessment must also take into account the unique qualities of metals: they are natural substances, some are essential for organism health, most organisms can regulate essential metals, and speciation and water–sediment chemistry govern bioavailability and hence, ultimately, toxicity.

Bulk sediment metals concentrations measured at one site will not necessarily be similarly bioavailable as the same metals concentrations measured in sediments at another site (Allen 1996). Although broad comparisons based on bulk sediment metals concentrations can be useful for hazard identification, risk assessment must recognize and allow for the complex interactions of metals in the aquatic environment that control their bioavailability and hence their potential risk (as previously discussed).

Hazard identification

Hazard identification evaluates the inherent potential for a given substance to produce an adverse biological effect. It also focuses on overall data consistency, nature of any effects observed, relevance of any effects to the ecosystem, mechanisms of action (if known), and patterns of dose–

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response relationships. Subsequent dose–response assessment provides a quantitative evaluation of the relationship between dose or exposure and severity of effects.

Hazard identification of metals depends primarily on identifying toxic responses (both acute and chronic). Ideally, toxicity testing or other means of directly assessing bioavailability should be conducted, as previously discussed. Alternatively, hazard can be evaluated by a process that some might consider exposure assessment and others might consider risk characterization. Specifically, bioavailable metal (whether from water (interstitial or overlying) or food) can probably be predicted, to the extent necessary for hazard identification, by comparing sediment metals concentrations with sediment quality values in a screening process. There are a variety of such values, with no consensus as to which is most appropriate for generic use. A weight of evidence approach should be used, comparing sediment metals concentrations with available, recognized sediment quality values (e.g., Long et al. 1995; Smith et al. 1996; U.S. EPA 1996). If none of the generic sediment quality values are exceeded, given that most values are conservative and protective, there is little or no hazard and also little or no risk. If all of the values are exceeded, there is a hazard. Intermediate responses may or may not indicate a hazard depending on how many responses are exceeded and by how much and how the sediment quality values were derived. Risk in the case of exceedances can only be determined by completing the remaining ERA steps. Best professional judgement erring on the side of conservatism is needed to make this assessment (cf. Menzie et al. 1996). Also, when making this assessment, particularly when using sediment quality values based on total metals concentrations, it must be remembered that metal speciation governs bioavailability. Thus, although this assessment can be useful, particularly when other data are not available, it serves only for screening and, even with inclusion of appropriate chemical normalization but without toxicity testing, it is far from definitive.

Dose–response assessment

Dose–response assessment is performed to determine the range and nature of the relationship between the dose and the resulting response. It is also conducted to identify the threshold where effects can be measured. The shape of the dose–response curve will vary depending on whether metals are essential (e.g., Cu, Zn) or nonessential (e.g., Hg). This curve will also shift depending on environmental parameters (e.g., salinity, pH, organic matter, particle size), which need to be characterized both by experimentation (e.g., in the laboratory) and by field observation, if possible.

Testing should involve both acute and chronic endpoints with test organisms that are not deficient in essential metals. Chronic tests are preferable and more accurate than bioaccumulation measurements for assessing the potential for long-term effects of metals in sediments. Testing should encompass both "worst-case" naturally relevant conditions (to be protective, bioavailability should be optimized, although not to an unreasonable extent, in order to provide the best possible evidence of "no effects" if such are determined) and "normal" or "expected" conditions (in order to provide evidence of what may actually happen if effects are determined).

Bioaccumulation measurements presently only provide evidence of bioavailability for specific chemicals and as a surrogate for possible effects via ingestion of prey (U.S. EPA/ACOE 1994). Such measurements should involve field and laboratory analyses of tissue concentrations following sediment exposures. Bioaccumulation data are metal and organism specific and presently not easily interpreted relative to possible adverse effects unless they can be linked to direct effects or to transfer to higher trophic levels (Chapman 1997*c*). However, when good information is available on concentration–toxicity relationships, bioaccumulation data may be the most useful of all data for linking cause and effect.

Exposure assessment

Exposure assessment measures the magnitude and distribution of metals in sediment. Precise and accurate measurements are required; exposure assessments will improve as analytical and sampling methods improve. Key aspects of such measurements (e.g., speciation, AVS/SEM) have been fully discussed and only briefly summarized below.

Metal-binding phases in sediments (e.g., AVS, POC, FeOOH, MnOOH) reduce metals availability and hence exposure. In contrast, complexation by ligands in interstitial water may enhance metal mobilization but often lowers bioavailability (Allen and Hansen 1996) as does sediment oxidation of metal-binding phases. Both analytical techniques for measuring metal speciation related to bioavailability and thermodynamic calculations suffer from technique-specific limitations. Empirical estimates of exposure include chemical concentrations (in whole sediment, porewater, overlying water, SEM, tissues) and biomarkers. Modelled/calculated estimates of exposure include normalized sediment concentrations, fate and transfer models, and food chain models. Experimental or field data are more useful than model calculations.

Exposure assessments must consider the complexity of metals speciation in the environment, transformations, and interactions between different metals and metal species and between metal-binding phases in sediments. These factors all affect bioavailability. For instance, in situ metals toxicity in sediments may be far less in tests carried out under appropriate physicochemical conditions than under standardized laboratory testing conditions. For many substances, including metals, mixtures toxicity can be addressed relatively simply assuming additivity (Pederson and Petersen 1996).

All species of a metal are not the same and should not be treated as such. Opportunity for exposure will vary for different species of the same metal. The most important factor controlling exposure is speciation of metals in sediment phases. As previously noted, not all metal forms are equally bioavailable. Other key factors affecting bioavailability include potential for release by oxidation of sulfides or complexation and possible dissolution after ingestion. Also, although exposure may ultimately result in a risk from nonessential metals, this is not the case for essential metals. Lack of exposure indicates no risk from excess metal (although deficiency may occur for essential metals). A decision tree and modifications to traditional risk assessment, for essential metals, are provided in ICME (1997) to address the

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issue of deficiency (which is common in terrestrial but probably not in sedimentary environments).

Organisms may also modify exposure. For example, polychaetes can irrigate their burrows with overlying water, avoiding contact with porewater (Miron and Kristensen 1993). Meiofaunal bioturbation can affect partitioning of Cd between porewater and sediment, resulting in a significant increase in porewater Cd concentrations (Green and Chandler 1994). Emerging insects can remobilize and export Cd from lake sediments (Currie et al. 1997).

Both the PEC and the PNEC of a metal or metal species should be based on similar levels of availability (i.e., metal/metal species) because availability for uptake in the natural environment can be much less than in the laboratory environment. Speciation needs to be taken into account to the extent possible given presently available analytical and sampling methods and understanding of metal dynamics in sediments. PNECs should not be below natural background levels, especially for essential metals.

Risk characterization

The focus here as elsewhere in this paper is on metals in sediments. Information on generic tools such as the quotient method, Bayesian/Monte Carlo probabilistic assessments, etc., is provided in HERA (1996*b*) and Ingersoll et al. (1997).

Risk depends on the relationship between existing or future exposure and potential effects and the co-occurrence of the exposure in the organisms of interest. Risk should consider background or "natural" conditions. Since minerals are natural compounds, "pollution" in highly mineralized areas may not be anthropogenic. Similarly, not all aquatic environments have the same background or natural metal levels. Further, although many hazard and risk assessment techniques consider contaminants individually, contaminants rarely occur singly in nature.

Adaptation of organisms to elevated metals concentrations in sediments is well documented (Wentsel et al. 1978; Pesch and Hoffman 1982). It can occur relatively rapidly for dominant benthic infauna such as worms and insects (Klerks and Levinton 1989, 1992; Miller and Hendricks 1996), is genetically determined (Klerks and Weis 1987; Klerks and Bartholomew 1991; Hoare et al. 1995), and may occur relatively simply without appreciable cost to the organism (Martinez and Levinton 1996). The reality of adaptation must be considered, i.e., that field populations may show no effects from elevated metals concentrations in sediments when laboratory studies indicate toxicity (e.g., Correa et al. 1996). Organisms do not adapt to the other end of the toxicity curve, e.g., to deficiency.

The most definitive means for reconciling or balancing the multiple lines of evidence that must be assessed during the risk characterization phase of an ERA is weight of evidence (Chapman 1996*b*; Menzie et al. 1996). Suggestions for evaluating the importance of different lines of evidence are provided in U.S. EPA (1997). The results of a weight of evidence approach should not be reported as a single point estimate of risk, which provides an appearance of precision that does not exist, but rather should be presented as a range, including descriptions of the assumptions and uncertainties behind these values.

Because contaminated sediments typically contain a complex mixture of contaminants (e.g., not only one metal and generally not only metals), an initial screening-level risk characterization should be performed to assess the potential for risk from sediment contaminants. If there is no risk, there is no further need to assess sediment contaminants. However, if there is risk, then evaluation is needed. Possible causative agents can be suggested based on correlations between elevated sediment concentrations and adverse effects (in the laboratory, in the field, or both, i.e., burden of evidence as discussed above). More definitive assessment may be possible in some cases where AVS and SEM data are available (thus, such data should be acquired). Specifically, when the SEM:AVS < 1, Cd, Cu, Ni, Pb, and Zn are not causing toxicity in the sediments (Chapman 1997*b*). When SEM exceeds AVS on a molar basis, toxic effects may be expressed. Weight of evidence should be used as recommended by Suter (1996) by comparing measured concentrations with different sediment quality values as suggested for hazard assessment and then examining ameliorating factors such as AVS/SEM (where appropriate), organic complexation, etc. More in-depth risk characterization can involve the use of spiked sediment toxicity tests (e.g., Murdoch et al. 1997) or sediment TIEs. Such tests and evaluations provide the means for more conclusively establishing whether toxicity is due to metals, other substances, or a combination of the two, i.e., for establishing cause and effect. TIEs have proven effective in determining toxicity due to specific metals, e.g., Cd, Ni, Cu, and Zn (Ankley and Schubauer-Berigan 1995). If toxicity is not due to metals in specific sediments, then other sediment contaminants are responsible for any ecological risk from those sediments. Determining tissue contaminant concentrations in toxicity tests can also provide information related to causality, although less so for essential than for nonessential metals (Chapman 1997*c*). Determining causality is, of course, critical for remediation, which can involve reducing existing sources and (or) reducing or eliminating exposure of biota to toxic sediments.

Remediation

Remediation decisions must be risk based and incorporate site-specific considerations due to the complex nature of metals–sediment interactions relative to hazard and, ultimately, risk (NRC 1997). In particular, the level of risk needs to be considered in conjunction with remediation alternatives so that any actions are not only appropriately environmentally protective but also cost-effective. Templeton and Della (1996) described a three-level risk-based approach to separate out sediments that are not of environmental concern from those that are, to a moderate or high extent, based on bioavailability.

Cleanup strategies and technologies are comprehensively reviewed in NRC (1997). Remediation alternatives include the following: natural recovery, enhanced natural recovery, capping, in situ treatment, dredging, and disposal (e.g., unconfined open-water disposal, confined disposal, uplands disposal which may include treatment).

Natural recovery (i.e., the "do nothing" option) is applicable when either risk is low from sediments in contact with biota or there is little risk of sediments coming into contact

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with biota (e.g., hazardous sediments are covered with cleaner material). It is also applicable where other options will increase rather than reduce the risk. Natural recovery assumes that sediments are being buried naturally or that otherwise (e.g., mixing with cleaner sediments, biodegradation) the bioavailability of metals is being reduced to non-hazardous levels within a reasonable period of time. It also assumes that sources of continuing or future contamination have been eliminated and that sediment will not be remobilized.

Enhanced natural recovery involves placing a thin layer of clean material over the contaminated sediment in such a way that populations of sediment-dwelling organisms are not destroyed.

Capping requires that the sediments being capped not be in an area requiring navigational or other dredging and that groundwater flow not be a major factor. As for natural recovery, assumptions include elimination of sources and that the cap will not be disturbed. Capping is not possible in all situations due to factors including currents, depth, and bottom topography.

Capping, natural recovery, or enhanced natural recovery are often the remediation strategies of choice. First, these are the lowest cost options. Second, the contaminated sediments are not disturbed. Therefore, there is not the additional input of particles and dissolved contaminants that accompanies dredging activities.

In situ treatment can involve solidification/stabilization, biological, or chemical treatment. Solidification/stabilization involves fixing or solidifying the sediments with reagents that bind contaminants to reduce their bioavailability. Similar constraints to capping apply, and this technique will increase the volume of existing sediments. However, it can be used for metals-contaminated sediments (Wardlaw 1995). Biological degradation of contaminants by aerobic or anaerobic treatment is not useful for metals-contaminated sediments. In contrast, some forms of chemical treatment are applicable to metals-contaminated sediments. Some of these procedures have been demonstrated in freshwaters but not in marine waters (NRC 1997).

Dredging is only warranted for sediments that are truly hazardous to the biota if left in place or if necessary for navigation. Special equipment or procedures may be required to reduce turbidity and resuspension during dredging of environmentally sensitive areas (Mohan 1996). Sediment disturbance will result in oxidation of anoxic sediments; however, field studies indicate that resulting metals mobilization (e.g., from dredging) is not significant (Hirst and Aston 1983; Slotten and Reuter 1995). Information on different dredging technologies is provided in Mohan (1996) and NRC (1997). Disposal options and the extent to which material is removed from contact with biota vary, depending on risk. Sediments with low risk are generally appropriate for unconfined open-water disposal; those with moderate to high risk are generally subject to confined disposal, which may be uplands disposal where there is a high risk if materials are not completely removed from the aquatic environment.

Uplands disposal may include ex situ treatment, e.g., incineration. However, this is only viable where sediments contain high concentrations of combustible contaminants, i.e., it is not applicable to sediments where metals are the major contaminant (Mohan 1996). Treatment can also include stabilization or solidification techniques, such as may also be used in situ, or washing (primarily for sands and gravels) to remove and treat contaminated porewater. Metal extraction technologies exist, are applicable to metals-contaminated sediments, and have been applied to Great Lakes sediments, as has washing (Wardlaw 1995). Encapsulation is a form of capping in which contaminated silty material is injected into a layer of clean silt so that contaminants such as metals are adsorbed and thus immobilized. However, since adsorption is a reversible process, continued immobilization depends on maintaining chemical equilibrium conditions.

Other upland treatment techniques include biological and chemical treatment and extraction technologies (reviewed in Mohan 1996; NRC 1997). Similar comments apply to biological and chemical treatment ex situ as in situ; in particular, most testing has been done in freshwater rather than marine environments. Many extraction technologies (e.g., the use of solvents) are not applicable to metals-contaminated sediments. Additionally, in marine sediments, high salt concentrations will affect sediment surface chemistry and metals ion-exchange capacity. To date, the technically most useful and lowest cost remediation approach other than dredging is fixation. Although solidification/stabilization is applicable to most metals-contaminated freshwater sediments, it has not been demonstrated in marine waters and is not accepted by many jurisdictions as an environmentally acceptable alternative because the metals are left in place and because of significant volume increases in the contaminated sediments (Wardlaw 1995). Other remediation technologies are expensive and (or) only applicable to a limited subset of sediment types. Thus, at present, there is no low-cost, universal metal treatment alternative that is also universally acceptable. However, many contaminated sediments can be managed effectively using natural recovery, capping, or containment (NRC 1997). These procedures are as applicable to metals as to other contaminants and equally applicable to fresh and marine waters.

Conclusions and future research needs

The key results of our literature review are presented along with recommendations for future research. In particular, research to date has focused on metals such as Cd, Pb, and Zn. Relatively little attention has been focused on metals such as Fe, Mn, Cr, and Sb or metalloids such as As and Se.

Sediment binding and release

Metal binding in sediments is controlled by FeOOH and MnOOH, organic matter, and sulfides, depending on the redox potential of the sediments. Ankley et al. (1996) have pointed out that there are two key issues in assessing metal bioavailability in sediments: temporal and spatial variations of AVS in sediments and practical, quantitative assessment of the significance of metal-binding phases other than AVS, particularly for aerobic systems. The AVS/SEM model predicts nontoxicity from metals-contaminated sediments but is only applicable for five divalent metals and only for anaero-

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bic sediments where AVS is present. Normalization by POC (both aerobic and anaerobic sediments) or FeOOH and MnOOH (aerobic sediments) has been used to assess the potential for toxicity; however, binding constants are not well understood. Metal mobilization will occur as a result of bioturbation and bioirrigation, oxidative dissolution, and dissolution facilitated by complexing ligands. Predictive approaches to metal release require an improved understanding of formation constants with polysulfides, thiols, and other natural ligands.

Bioavailability

Metal bioavailability is controlled by partitioning into porewaters. Highest porewater concentrations will be found in oxic sediment layers; bioavailability of metals in these waters will be related to the free metal ion concentration and complexation by organic matter in the porewaters. More information is required to reliably predict metal bioavailability. In the interim, methods are being pursued that measure speciation in porewaters, and these can be evaluated for their relevance by comparison with bioassays. Oxidation of anaerobic sediments containing MeS by physical (e.g., storms), biological (e.g., bioirrigation), and human activities (e.g., dredging, trawling) may result in the direct release of free metal ion. To predict such releases, which will be relatively minor for overlying waters but possibly not for porewaters, oxidation rates need to be quantified. Similarly, and related to thermodynamic calculations, there is a need to determine formation constants of metal complexes with ligands, sorption constants, and the crystalline forms of MeS and their solubility products. The FIAM is useful for predicting the bioavailable species, but other labile metal species and nonchemical variables also determine metal bioavailability. Carefully defined experiments on FIAM with organic ligands are particularly needed. Most studies determine soluble metal concentrations; instead, they should be focusing on bioavailable metal fractions such as free metal ion activities. Biotic site (e.g., gill) binding models have shown promise predicting toxicity to fish for systems of differing chemistry but have yet to be applied to marine systems or to benthic organisms.

Hazard identification

Laboratory or in situ toxicity (acute and chronic) testing with whole organisms in a test battery approach involving more than one test species and exposure route (e.g., porewater, overlying water, whole sediment) is the best predictor of hazard. Predictions based on sediment chemistry measurements are presently only useful for screening, not for final decision-making. It is not presently possible to predict or fully determine the consequences associated with bioaccumulation and food chain transfer of metals from sediments; thus, bioaccumulation is not presently useful for hazard identification, particularly of essential metals. The former issue relates to linking bioaccumulation with impacts (Chapman 1997*c*). The latter issue refers not to biomagnification, which only occurs in the case of metals for methylated Hg, but rather to the possibility of secondary poisoning. A better understanding of secondary poisoning effects is required for metals, in particular the importance of exposure via gut uptake.

ERA

Determining the risk posed by metals in sediments depends on determining bioavailability, whether from water (overlying, interstitial) or food. Such determinations can be estimated prospectively (normalized sediment chemistry, laboratory bioassays including biomarkers but especially whole-sediment exposures) or assessed retrospectively (e.g., in situ bioassays, field studies). At present the SEM:AVS ratio provides the only mechanistically based procedure for the prediction of metals sediment toxicity. Binding of metals by other sediment phases and chemical speciation of porewater need to be understood before sediment toxicity can be fully predicted without actual testing. Accurately determining risk to ecological communities requires an integrated approach such as the Triad, where conclusions are based on a weight of evidence. Although bioaccumulation must be considered, the emphasis should be on toxicity (acute and chronic endpoints with organisms that are not deficient in essential metals). In addition, ERA of metals-contaminated sediments needs to consider the three separate possible transformation processes for metals in the aquatic environment: solubilization (= increased bioavailability), association with sediments (= decreased bioavailability), and resolubilization (= increased bioavailability). Other considerations that particularly require further research include the differences between essential and nonessential metals, the fact that persistence in sediments tends to reduce bioavailability, the complex interactions that control bioavailability and that tend to be site and situation specific, adaptation, which may occur relatively simply without appreciable cost to the organism, sediment particle ingestion as a source of metals for some species, and causality related to possible remediation.

Remediation

Remediation decisions, if not risk based, may be inappropriate. Not all available remediation technologies are applicable to metals-contaminated sediments. Fixation appears to be useful but is not generally accepted, and other remediation methods are expensive and not generally applicable. Because the major impediment to acceptance of fixation (other than the volume increase) is concern regarding short- and long-term bioavailability and toxicity of the fixed material, the efficacy of fixation needs to be more fully examined. Remediation of metals-contaminated sediments in marine environments requires particular consideration. However, in many cases, natural recovery and capping provide the most effective and safest methods of remediation.

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References

Adams, D.D. 1991.Sediment porewater sampling. *In* CRC handbook of techniques for aquatic sediments sampling. *Edited by* A. Mudroch and S.D. MacKnight. CRC Press, Boca Raton, Fla. pp. 131–169.

Adams, W.J., Kimerle, R.A., and Barnett, J.W., Jr. 1992. Sediment quality and aquatic life assessment. Environ. Sci. Technol. **26**: 1864–1875.

Allen, H.E. 1996. Standards for metals should not be based on total concentration. Learned Discourse. Soc. Environ. Toxicol. Chem. News, **16**: 18–19.

Allen, H.E., and Hansen, D.J. 1996. The importance of trace metal speciation to water quality criteria. Water Environ. Res. **68**: 42–54.

Allen, H.E., Fu, G., and Deng, B. 1993. Analysis of acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) for estimation of potential toxicity in aquatic sediments. Environ. Toxicol. Chem. **12**: 1441–1453.

Allison, J.D., Brown, D.S., and Novo-Gradac, K.J. 1991. MINTEQA2/PRODEFA2, a geochemical assessment model for environmental systems: version 3.0 user's manual. U.S. Environmental Protection Agency, Athens, Ga.

Anderson, J., Birge, W., Gentile, J., Lake, J., Rodgers, J., Jr., and Swartz, R. 1987. Biological effects, bioaccumulation and ecotoxicology of sediment-associated chemicals. *In* Fate and effects of sediment-bound chemicals in aquatic systems. *Edited by* K.L. Dickson, A.W. Maki, and W.A. Brungs. Pergamon Press, New York. pp. 267–296.

Andreasson, M., and Dave, G. 1995. Transfer of heavy metals from sediments to fish, and their biliary excretion. J. Aquat. Ecosyst. Health, **4**: 221–230.

Ankley, G.T. 1996. Evaluation of metal/acid volatile sulfide relationships in the prediction of metal bioaccumulation by benthic macroinvertebrates. Environ. Toxicol. Chem. **15**: 2138–2146.

Ankley, G.T., and Schubauer-Berigan, M.K. 1995. Background and overview of current sediment toxicity identification evaluation procedures. J. Aquat. Ecosyst. Health, **4**: 133–149.

Ankley, G.T., Katko, A., and Arthur, J.W. 1990. Identification of ammonia as an important sediment associated toxicant in the Lower Fox River and Green Bay. Environ. Toxicol. Chem. **9**: 313–322.

Ankley, G.T., Phipps, G.L., Leonard, E.N., Benoit, D.A., Mattson, V.R., Kosian, P.A., Cotter, A.M., Dierkes, J.R., Hansen, D.J., and Mahony, J.D. 1991. Acid volatile sulfide as a factor mediating cadmium and nickel bioavailability in contaminated sediments. Environ. Toxicol. Chem. **10**: 1299–1307.

Ankley, G.T., Mattson, V.R., Leonard, E.N., West, C.W., and Bennett, J.L. 1993. Predicting the acute toxicity of copper in freshwater sediments: evaluation of the role of acid-volatile sulfide. Environ. Toxicol. Chem. **12**: 315–320.

Ankley, G.T., Thomas, N.A., Di Toro, D.M., Hansen, D.J., Mahony, J.D., Berry, W.J., Swartz, R.C., Hoke, R.A., Garrison, A.W., Allen, H.E., and Zarba, C.S. 1994. Assessing potential bioavailability of metals in sediments: a proposed approach. Environ. Manage. **18**: 331–337.

Ankley, G.T., Di Toro, D.M., Hansen, D.J., and Berry, W.J. 1996*b*. Technical basis and proposal for deriving sediment quality criteria for metals. Environ. Toxicol. Chem. **15**: 2056–2066.

ASTM. 1994. Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. E-1391-94. American Society for Testing and Materials, Philadelphia, Pa.

Balistrieri, L., Brewer, P.G., and Murray, J.W. 1981. Scavenging residence times of trace metals and surface chemistry of sinking particles in the deep ocean. Deep-Sea Res. **28A**: 101–121.

Becker, D.S., and Ginn, T.C. 1995. Effects of storage time on toxicity of sediments from Puget Sound, Washington. Environ. Toxicol. Chem. **14**: 829–835.

Belzile, N., De Vitre, R.R., and Tessier, A. 1989. In situ collection of diagenetic iron and manganese oxyhydroxides from natural sediments. Nature (Lond.), **340**: 376–377.

Benson, W.H., and Di Giulio, R.T. 1992. Biomarkers in hazard assessments of contaminated sediments. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 241–266.

Berry, W.J., Hansen, D.J., Mahony, J.D., Robson, D.L., Di Toro, D.M., Shipley, B.P., Rogers, B., Corbin, J.M., and Boothman, W.S. 1996. Predicting the toxicity of metal-spiked laboratory sediments using acid-volatile sulfide and interstitial water normalizations. Environ. Toxicol. Chem. **15**: 2067–2079.

Besser, J.M., Ingersoll, C.G., and Giesy, J.P. 1996*a*. Effects of spatial and temporal variation of acid-volatile sulfide on the bioavailability of copper and zinc in freshwater sediments. Environ. Toxicol. Chem. **15**: 286–293.

Besser, J.M., Giesy, J.P., Kubitz, J.A., Verbrugge, D.A., Coon, T.G., and Braselton, W.E. 1996*b*. Assessment of sediment quality in dredged and undredged areas of the Trenton channel of the Detroit River, Michigan, USA, using the sediment quality triad. J. Gt. Lakes Res. **22**: 683–696.

Beyer, J.M., Sandvik, K., Hylland, E., Fjeld, E., Egaas, E., Aas, E., Skare, J.U., and Goksoyr, A. 1994. Contaminant accumulation and biomarker responses in flounder (*Platichthys flesus* L.) and Atlantic cod (*Gadus morhua* L.) exposed by caging to polluted sediments in Sorfjorden, Norway. Aquat. Toxicol. **36**: 75–98.

Borgmann, U., and Norwood, W.P. 1997*a*. Toxicity and accumulation of zinc and copper in *Hyalella azteca* exposed to metal-spiked sediments. Can. J. Fish. Aquat. Sci. **54**: 1046–1054.

Borgmann, U., and Norwood, W.P. 1997*b*. Identification of the toxic agent in metal-contaminated sediments from Manitouwadge Lake, Ontario, using toxicity–accumulation relationships in *Hyalella azteca*. Can. J. Fish. Aquat. Sci. **54**: 1055–1063.

Boulegue, J. 1983. Trace metals (Fe, Cu, Zn, Cd) in anoxic environments. *In* Trace metals in sea water. *Edited by* C.S. Wong, E. Boyle, K.W. Bruland, and J.D. Burton. Plenum Press, New York. pp. 563–577.

Brendel, P.J., and Luther, G.W. 1995. Development of a gold amalgam voltammetric microelectrode for the determination of dissolved Fe, Mn, O_2, and S(–II) in porewaters of marine and freshwater sediments. Environ. Sci. Technol. **29**: 751–761.

Brouwer, H., and Murphy, T.P. 1994. Diffusion method for the determination of acid-volatile sulfide (AVS) in sediment. Environ. Toxicol. Chem. **13**:1273–1275

Bryan, G.W. 1985. Bioavailability and effects of heavy metals in marine deposits. *In* Wastes in the sea. Vol. 6. *Edited by* B. Ketchum, J. Capuzzo, W. Burt, I. Duedall, P. Park, and D. Kester. John Wiley & Sons, Inc., New York. pp. 41–56.

Bufflap, S.E., and Allen, H.E. 1995. Sediment porewater collection method for trace metal analysis: a review. Water Res. **29**: 165–177.

Burgess, R.M., Rogers, B.A., Rego, S.A., Corbin, J.M., and Morrison, G.E. 1994. Sand spiked with copper as a reference toxicant material for sediment toxicity testing: a preliminary evaluation. Arch. Environ. Contam. Toxicol. **26**: 163–168.

Burton, G.A., Jr. 1992*a*. Sediment collection and processing: factors affecting realism. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 37–66.

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Burton, G.A., Jr. 1992*b*. Plankton, macrophyte, fish and amphibian toxicity testing of freshwater sediments. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 167–182.

Burton, G.A., Jr., and Scott, K.J. 1992. Sediment toxicity evaluations: their niche in ecological assessments. Environ. Sci. Technol. **26**: 2068–2075.

Burton, G.A., Jr., Drotar, A., Lazorchak, J.M., and Bahls, L.L. 1987. Relationship of microbial activity and *Ceriodaphnia* responses to mining impacts on the Clark Fork River, Montana. Arch. Environ. Contam. Toxicol. **16**: 523–530.

Burton, G.A., Jr., Nelson, M.K., and Ingesoll, C.G. 1992. Freshwater benthic toxicity tests. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 213–240.

Cai, W.J., and Reimers, C.E. 1993. The development of pH and pCO_2 microelectrodes for studying the carbonate chemistry of porewaters near the sediment–water interface. Limnol. Oceanogr. **38**: 1762–1773.

Cairns, J., Jr. 1985. Multispecies toxicity testing. Pergamon Press, Elmsford, N.Y.

Cairns, J., Jr., Niederlehner, B.R., and Smith, E.P. 1992. The emergence of functional attributes as endpoints in ecotoxicology. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 111–128.

Campbell, P.G.C. 1995. Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. *In* Metal speciation and bioavailability in aquatic systems. *Edited by* A. Tessier and D.R. Turner. John Wiley & Sons, Inc., New York. pp. 45–102.

Campbell, P.G.C., and Tessier, A. 1994. Geochemistry and bioavailability of trace metals in sediments. *In* Aquatic ecotoxicology: fundamental concepts and methodologies. Vol. 1. *Edited by* A. Boudou and F. Ribeyre. CRC Press, Boca Raton, Fla. pp. 125–148.

Canfield, T.J., Kemble, N.E., Grumbaugh, W.G., Dyer, F.J., Ingersoll, C.G., and Fairchild, J.F. 1994. Use of benthic invertebrate community structure and the sediment quality triad to evaluate metal-contaminated sediment in the Upper Clark Fork River, Montana. Environ. Toxicol. Chem. **13**: 1999–2012.

Canfield, T.J., Dyer, F.J., Fairchild, J.F., Haverland, P.S., Ingersoll, C.G., Kemble, N.E., Mount, D.R., La Point, T.W., Burton, G.A., and Swift, M.S. 1996. Assessing contamination in Great Lakes sediments using benthic communities and the sediment quality triad approach. J. Gt. Lakes Res. **22**: 565–583.

Carigan, R. 1984. Interstitial water sampling by dialysis: methodological notes. Limnol. Oceanogr. **29**: 667–670.

Carigan, R., Rapin, F., and Tessier, A. 1985. Sediment porewater sampling for metal analysis: a comparison of techniques. Geochim. Cosmochim. Acta, **49**: 2493–2497.

Carlson, A.R., Phipps, G.L., Mattson, V.R., Kosian, P.A., and Cotter, A.M. 1991. The role of acid volatile sulfide in determining cadmium bioavailability in sediments. Environ. Toxicol. Chem. **10**: 1309–1319.

Casas, A.M., and Crecelius, E.A. 1994. Relationship between acid volatile sulfide and the toxicity of zinc, lead and copper in marine sediments. Environ. Toxicol. Chem. **13**: 529–436.

Chapman, P.M. 1988. Marine sediment toxicity tests. *In* Biological and chemical characterization of sludges, sediments, dredge spoils, and drilling muds. *Edited by* J.J. Lichtenberg, F.A. Winter, C.I. Weber, and L. Fradkin. Spec. Tech. Publ. 976, American Society for Testing and Materials, Philadelphia, Pa. pp. 391–402.

Chapman, P.M. 1996*a*. Hazard identification, hazard classification and risk assessment for metals and metal compounds in the aquatic environment. International Council on Metals and the Environment, Ottawa, Ont.

Chapman, P.M. 1996*b*. Presentation and interpretation of Sediment Quality Triad data. Ecotoxicology, **5**: 327–339.

Chapman, P.M. 1997*a*. Death by mud: amphipod sediment toxicity tests. *In* Microscale aquatic toxicology — advances, techniques and practice. *Edited by* P.G. Wells, K. Lee, and C. Blaise. CRC Press, Boca Raton, Fla. pp. 451–463.

Chapman, P.M. 1997*b*. Acid volatile sulfides, equilibrium partitioning, and hazardous waste site sediments. Environ. Manage. **21**: 197–201.

Chapman, P.M. 1997*c*. Is bioaccumulation useful for predicting impacts? Mar. Pollut. Bull. **34**: 282–283.

Chapman, P.M., Power, E.A., and Burton, G.A., Jr. 1992. Integrative assessments in aquatic ecosystems. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 313–340.

Chapman, P.M., Allen, H.E., Godtfredsen, K., and Z'Graggen, M.N. 1996*a*. Evaluation of bioaccumulation factors in regulating metals. Environ. Sci. Technol. **30**: 448A–452B.

Chapman, P.M., Thornton, I., Persoone, G., Janssen, C., Godtfredsen, K., and Z'Graggen, M.N. 1996*b*. International harmonization related to persistence and bioavailability. Hum. Ecol. Risk Assess. **2**: 393–404.

Chapman, P.M., Fairbrother, A., and Brown, D. 1998. A critical evaluation of safety (= uncertainty) factors for ecological risk assessment. Environ. Toxicol. Chem. **17**: 99–108.

Chappie, D.J., and Burton, G.A., Jr. 1997. Optimization of in situ bioassays with *Hyalella azteca* and *Chironomus tentans*. Environ. Toxicol. Chem. **16**: 559–564.

Correa, J.A., Gonzalez, P., Sanchez, P., Munoz, J., and Orellana, M.C. 1996. Copper–algae interactions: inheritance or adaptation? Environ. Monit. Assess. **40**: 41–54.

Cowan, C.E., Jenne, E.A., and Kinnison, R.R. 1986. Methodology for determining the relationship between toxicity and the aqueous speciation of a metal. *In* Aquatic toxicity and environmental fate. Vol. 9. *Edited by* T.M. Poston and R. Purdy. Spec. Tech. Publ. 921, American Society for Testing and Materials, Philadelphia, Pa. pp. 463–478.

Currie, R.S., Fairchild, W.L., and Muir, D.C.G. 1997. Remobilization and export of cadmium from lake sediments by emerging insects. Environ. Toxicol. Chem. **16**: 2332–2338.

Davis, J.A. 1984. Complexation of trace metals by adsorbed natural organic matter. Geochim. Cosmochim. Acta, **48**: 679–691.

Davis-Colley, R.J., Nelson, P.O., and Williamson, K.J. 1984. Copper and cadmium uptake by estuarine sedimentary phases. Environ. Sci. Technol. **18**: 491–499.

Davison, W., and Zhang, H. 1994. In situ speciation measurements of trace components in natural waters using thin-film gels. Nature (Lond.), **367**: 546–548.

Davison, W., Grime, G.W., Morgan, J.A.W., and Clarke, K. 1991. Distribution of dissolved iron in sediment porewaters at submillimetre resolution. Nature (Lond.), **352**: 323–325.

Dawson, D.A., Stebler, E.F., Burks, S.L., and Bantle, J.A. 1988. Evaluation of the developmental toxicity of metal-contaminated sediments using short-term fathead minnow and frog embryo–larval assays. Environ. Toxicol. Chem. **7**: 27–34.

Deaver, E., and Rodgers, J.H., Jr. 1996. Measuring bioavailable copper using anodic stripping voltammetry. Environ. Toxicol. Chem. **15**: 1925–1930.

Decaprio, A.P. 1997. Biomarkers: coming of age for environmental health and risk assessment. Environ. Sci. Technol. **31**: 1837–1848.

© 1998 NRC Canada

De Witt, T.H., Swartz, R.C., Hansen, D.J., McGovern, D., and Berry, W.J. 1996. Bioavailability and chronic toxicity of cadmium in sediment to the estuarine amphipod, *Leptocheirus plumulosus*. Environ. Toxicol. Chem. **15**: 2095–2101.

Diaz, R.J. 1992. Ecosystem assessment using estuarine and marine benthic community structure. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 67–86.

Di Toro, D.M., Mahony, J.D., Hansen, D.J., Scott, K.J., Hicks, M.B., Mayr, S.M., and Redmond, M.S. 1990. Toxicity of cadmium in sediments: the role of acid volatile sulfide. Environ. Toxicol. Chem. **9**: 1487–1502.

Di Toro, D.M., Zarba, C.S., Hansen, D.J., Berry, W.J., Swartz, R.C., Cowan, C.E., Pavlou, S.P., Allen, H.E., Thomas, N.A., and Paquin, P.R. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environ. Toxicol. Chem. **10**: 1541–1583.

Di Toro, D.M., Mahony, J.D., Hansen, D.J., Scott, K.J., Carlson, A.R., and Ankley, G.T. 1992. Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. Environ. Sci. Technol. **26**: 96–101.

Di Toro, D.M., Mahony, J.D., and Gonzalez, A.M. 1996*a*. Particle oxidation model of synthetic FeS and sediment AVS. Environ. Toxicol. Chem. **15**: 2156–2167.

Di Toro, D.M., Mahony, J.D., Hansen, D.J., and Berry, W.J. 1996*b*. A model of the oxidation of iron and cadmium sulfide in sediments. Environ. Toxicol. Chem. **15**: 2168–2186.

Donat, J.R., and Bruland, K.W. 1990. A comparison of two voltammetric techniques for determining zinc speciation in northeast Pacific Ocean waters. Mar. Chem. **28**: 301–323.

Dzombak, D., and Morel, F.M.M. 1990. Surface complexation modeling: hydrous ferric oxide. John Wiley & Sons, New York.

Elliott, M., and McLusky, D.S. 1985. Invertebrate production ecology in relation to estuarine quality management. *In* Estuarine management and quality assessment. *Edited by* W. Halcrow and J.G. Wilson. Plenum Press, New York. pp. 85–104.

Erickson, R.J., Benoit, D.A., Mattson, V.R., Nelson, H.P., and Leonard, E.N. 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. Environ. Toxicol. Chem. **15**: 181–193.

Fisher, N.S. 1986. On the reactivity of metals for marine phytoplankton. Limnol. Oceanogr. **31**: 443–449.

Fisher, N.S., Teyssier, J.-L., Fowler, S.W., and Wang, W.-X. 1996. Accumulation and retention of metals in mussels from food and water: a comparison under field and laboratory conditions. Environ. Sci. Technol. **30**: 3232–3242.

Fleming, R., Maund, S., and Murray, L. 1997. Ecological risk assessment for sediments: a European perspective. *In* Ecological risk assessment of contaminated sediments. *Edited by* G.C. Ingersoll, T. Dillon, and G.R. Biddinger. SETAC Press, Pensacola, Fla. pp. 367–376.

Forstner, U. 1982. Accumulative phases for heavy metals in limnic sediments. Hydrobiologia, **91**: 269–284.

Förstner, U. 1995. Non-linear release of metals from aquatic sediments. *In* Biogeodynamics of pollutants in soils and sediments: risk assessment of delayed and non-linear responses. *Edited by* W. Salomons and W.M. Stigliani. Springer-Verlag, Berlin. pp. 247–307.

Fu, G., Allen, H.E., and Cao, Y. 1992. The importance of humic acids to proton and cadmium binding in sediments. Environ. Toxicol. Chem. **11**: 1363–1372.

Gagnon, C., Mucci, A., and Pelletier, E. 1996. Vertical distribution of dissolved sulphur species in coastal marine sediments. Mar. Chem. **52**: 195–209.

Giesy, J.P., and Hoke, R.A. 1989. Freshwater sediment toxicity bioassessment: rationale for species selection and test design. J. Gt. Lakes Res. **15**: 539–569.

Giesy, J.P., Graney, R.L., Newsted, J.L., Rosiu, C.J., Benda, A., Kries, R.G., Jr., and Horvath, F.J. 1988. Comparison of three sediment bioassay methods using Detroit River sediments. Environ. Toxicol. Chem. **7**: 483–498.

Giesy, J.P., Rosiu, C.J., Graney, R.L., and Henry, M.G. 1990. Benthic invertebrate bioassays with toxic sediment and porewater. Environ. Toxicol. Chem. **9**: 233–248.

Ginn, T.C., and Pastorok, R.A. 1992. Assessment and management of contaminated sediments in Puget Sound. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 371–402.

Gonzalez, A.M. 1996. A laboratory-formulated sediment incorporating synthetic acid-volatile sulfide. Environ. Toxicol. Chem. **15**: 2209–2220.

Green, A.S., and Chandler, G.T. 1994. Meiofanual bioturbation effects on the partitioning of sediment-associated cadmium. J. Exp. Mar. Biol. Ecol. **180**: 59–70.

Hamoutene, D., Romeo, M., Gnassia, M., and Lafaurie, M. 1996. Cadmium effects on oxidative metabolism in marine seagrass: *Posidonia oceanica*. Bull. Environ. Contam. Toxicol. **56**: 237–334.

Hansen, D.J., Berry, W.J., Mahony, J.D., Boothman, W.S., Di Toro, D.M., Robson, D.L., Ankley, D.L., Ma, D., Yan, Q., and Resch, D.E. 1996*a*. Predicting the toxicity of metal-contaminated field sediments using acid-volatile sulfide and interstitial water normalizations. Environ. Toxicol. Chem. **15**: 2080–2094.

Hansen, D.J., Mahony, J.D., Berry, W.J., Benyi, S.J., Corbin, J.M., Pratt, S.D., Di Toro, D.M., and Able, M.B. 1996*b*. Chronic effect of cadmium in sediments on colonization by benthic marine organisms: an evaluation of the role of interstitial cadmium and acid-volatile sulfide in biological availability. Environ. Toxicol. Chem. **15**: 2126–2137.

Hare, L., and Tessier, A. 1996. Predicting animal cadmium concentrations in lakes. Nature (Lond.), **380**: 430–432.

Hare, L., Carignan, R., and Huerta-Diaz, M.A. 1994. A field study of metal toxicity and accumulation by benthic invertebrates; implications for the acid-volatile sulfide (AVS) model. Limnol. Oceanogr. **39**: 1653–1668.

Harkey, G.A., Landrum, P.F., and Klaine, S.J. 1994. Comparison of whole-sediment, elutriate and pore-water exposures for use in assessing sediment-associated organic contaminants in bioassays. Environ. Toxicol. Chem. **13**: 1315–1329.

Harrahy, E.A., and Clements, W.H. 1997. Toxicity and bioaccumulation of a mixture of heavy metals in *Chironomus tentans* (Diptera:Chironomidae) in synthetic sediment. Environ. Toxicol. Chem. **16**: 317–327.

HERA. 1996*a*. The role of biomarkers in risk assessment. Debate/commentary. Hum. Ecol. Risk Assess. **2**: 243–274.

HERA. 1996*b*. Special issue: commemoration of the 50th anniversary of Monte Carlo. Hum. Ecol. Risk Assess. **2**: 627–1034.

Herbelin, A.L., and Westall, J.C. 1996. FITEQL — a computer program for determination of chemical equilibrium constants from experimental data. Rep. 96-01, Department of Chemistry, Oregon State University, Corvallis, Oreg.

Hirst, J.M., and Aston, S.R. 1993. Behaviour of copper, zinc, iron and manganese during experimental resuspension and reoxidation of polluted anoxic sediments. Estuarine Coastal Shelf Sci. **16**: 549–558.

Hoare, K., Beaumont, A.R., and Davenport, J. 1995. Variation among populations in the resistance of *Mytilus edulis* embryos

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to copper: adaptation to pollution? Mar. Ecol. Prog. Ser. **120**: 155–161.

Hoke, R.A., Giesy, J.P., Ankley, G.T., Newsted, J.L., and Adams, J.R. 1990. Toxicity of sediments from western Lake Erie and Maumee River at Toledo, Ohio, 1987: implications for current dredged material disposal practices. J. Gt. Lakes Res. **16**: 457–470.

Huerta-Diaz, M.A., Carignan, R., and Tessier, A. 1993. Measurement of trace metals associated with acid volatile sulfides and pyrite in organic freshwater sediments. Environ. Sci. Technol. **27**: 2367–2372.

Huerta-Diaz, M.A., Tessier, A., and Carignan, R. 1998. Geochemistry of trace metals associated with reduced sulfur in freshwater sediments. Appl. Geochem. **13**: 213–233.

Huggett, R.J., Kimerle, R.A., Merle, P.M., and Bergman, H.L. 1992. Biomarkers: biochemical, physiological and histopathological markers of anthropogenic stress. Lewis Publishers, Boca Raton, Fla.

Hulbert, M.H., and Brindle, M.P. 1975. Effects of sample handling on the composition of marine sedimentary porewater. Geol. Soc. Am. Bull. **86**: 19–110.

Hylland, K., Haux, C., and Hogstrand, C. 1992. Hepatic metallothionein and heavy metals in dab *Limanda limanda* from the German Bight. Mar. Ecol. Prog. Ser. **91**: 89–96.

ICME. 1997. International workshop on risk assessment of metals and their inorganic compounds. International Council on Metals and the Environment, Ottawa, Ont.

Ingersoll, C.G., Ankley, G.T., Benoit, D.A., Brunson, E.L., Burton, G.A., Dwyer, F.J., Hoke, R.A., Landrum, P.F., Norberg-King, T.J., and Winger, P.V. 1995. Toxicity and bioaccumulation of sediment-associated contaminants using freshwater invertebrates: a review of methods and applications. Environ. Toxicol. Chem. **14**: 1885–1894.

Ingersoll, C.G., Dillon, T., and Biddinger, G.R. 1997. Ecological risk assessment of contaminated sediments. SETAC Press, Pensacola, Fla.

Janes, N., and Playle, R.C. 1995. Modeling silver binding to gills of rainbow trout *Oncorhynchus mykiss*. Environ. Toxicol. Chem. **14**: 1847–1858.

Janssen, C.R., Vangheluwe, M.L., and Persoone, G. 1995. A multifactorial study of the effect of collection, storage and extraction techniques on the toxicity of freshwater sediments. Abstract Book, Soc. Environ. Toxicol. Chem. Second World Congress, SETAC Press. p. 264.

Jeroschewski, P., Steuckart, C., and Kühl, M. 1996. An amperometric microsensor for the determination of H_2S in aquatic environments. Anal. Chem. **68**: 4351–4357.

Kemp, P.F., and Swartz, R.C. 1986. Acute toxicity of interstitial and particle-bound cadmium to a marine infaunal amphipod. Mar. Environ. Res. **26**: 135–153.

Kheboian, C., and Bauer, C.F. 1987. Accuracy of selective extraction procedures for metal speciation in model aquatic sediments. Anal. Chem. **59**: 1417–1423.

Klerks, P.L., and Bartholomew, P.R. 1991. Cadmium accumulation and detoxification in a Cd-resistant population of the oligochaete *Limnodrilus hoffmeisteri*. Aquat. Toxicol. **19**: 97–112.

Klerks, P.L., and Levinton, S.L. 1989. Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site. Biol. Bull. **176**: 135–141.

Klerks, P.L., and Levinton, S.L. 1992. Evolution of resistance to metals in oligochaetes. *In* Ecotoxicology of metals in invertebrates. *Edited by* R. Dallinger and P. Rainbow. Lewis Publishers, Chelsea, Mich. pp. 223–241.

Klerks, P.L., and Weis, J.S. 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. Environ. Pollut. **45**: 173–205.

Koryta. 1990. Theory and application of ion selective electrodes, VIII. Anal. Chim. Acta, **233**: 1–31.

Lamberson, J.O., and Swartz, R.C. 1988. Use of bioassays in determining the toxicity of sediment to benthic organisms. *In* Toxic contaminants and ecosystem health: a Great Lakes focus. *Edited by* M.S. Evans. John Wiley & Sons, Inc., New York. pp. 257–279.

Lamberson, J.O., DeWitt, T.H., and Swartz, R.C. 1992. Assessment of sediment toxicity to marine benthos. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 183–212.

Landrum, P.F., Nihart, S.R., Eadie, B.J., and Herche, L.R. 1987. Reduction in bioavailability of organic contaminants to the amphipod *Pontoporeia hoyi* by dissolved organic matter of sediment interstitial waters. Environ. Toxicol. Chem. **6**: 11–20.

La Point, T.W., and Fairchild, J.F. 1992. Evaluation of sediment contaminant toxicity: the use of freshwater community structure. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 87–110.

Lee, H. 1992. Models, muddles and mud: predicting bioaccumulation of sediment-associated pollutants. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 367–294.

Leonard, E.N., Cotter, A.M., and Ankley, G.T. 1996. Modified diffusion method for analysis of acid volatile sulfide and simultaneously extracted metals in freshwater sediment. Environ. Toxicol. Chem. **15**: 1479–1481.

Liber, K., Call, D.J., Markee, T.P., Schmude, K.I., Balcer, M.D., Whiteman, F.W., and Ankley, G.T. 1996. Effects of acid-volatile sulfide on zinc bioavailability and toxicity to benthic macroinvertebrates: a spiked-sediment field experiment. Environ. Toxicol. Chem. **15**: 2113–2125.

Long, E.R., MacDonald, D.D., Smith, S.L., and Calder, F.D. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manage. **19**: 81–97.

Luoma, S.N. 1995. Prediction of metal toxicity in nature from bioassays: limitations and research needs. *In* Metal speciation and bioavailability in aquatic systems. *Edited by* A. Tessier and D.R. Turner. John Wiley & Sons Ltd., Chichester, U.K. pp. 609–660.

Luther, G.W., III. 1995. Trace metal chemistry in porewaters. *In* Metal contaminated aquatic sediments. *Edited by* H.E. Allen. Ann Arbor Press, Chelsea, Mich. pp. 65–80.

Luther, G.W., III, and Ferdelman, T.G. 1993. Voltammetric characterization of iron(II) sulfide complexes in laboratory solutions and in marine waters and porewaters. Environ. Sci. Technol. **27**: 1154–1163.

Luther, G.W., III, Rickard, D.T., Theberge, S., and Olyord, A. 1996. Determination of metal (bi)sulfide stability constants of Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} by voltammetric methods. Environ. Sci. Technol. **30**: 671–679.

Mac, M.J., and Smith, C.J. 1992. Sediment bioaccumulation testing with fish. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 295–312.

MacCrehan, W., and Shea, D. 1995. Temporal relationship of thiols to inorganic sulfur compounds in anoxic Chesapeake Bay sediment porewater. *In* Geochemical transformations of sedimentary sulfur. *Edited by* M.A. Vairavamurthy and M.A.A. Schoonen. Am. Chem. Soc. Symp. Ser. 612, American Chemical Society, Washington, D.C. pp. 294–310.

© 1998 NRC Canada

Mackey, D. 1982. Cation-exchange behavior of a range of absorbents and chromatographic supports with regard to their suitability for investigating trace metal speciation in natural waters. J. Chromatogr. **242**: 275–287.

MacRae, R.K. 1994. The copper binding affinity of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) gills. M.S. thesis, University of Wyoming, Laramie, Wyo.

Mahony, J.D., Di Toro, D., Gonzalez, A.M., Curto, M., Dilg, M., De Ross, L.D., and Sparrow, L.A. 1996. Partitioning of metals to sediment organic carbon. Environ. Toxicol. Chem. **15**: 2187–2197.

Mahony, J.D., Triveli, D., Santos, L., and Page, J. 1997. The role of AVS and organic carbon in partitioning of silver to sediments. Proceedings, 5th International Argentum Conference, Sept. 28 – Oct. 1, Hamilton, Ont.

Malcolm, S.J., Kershaw, P.J., Lovett, M.B., and Harvey, B.R. 1990. The interstitial water chemistry of ^{239,240}Pu and ^{241}Am in sediments of the northeast Irish Sea. Geochim. Cosmochim. Acta, **54**: 29.

Malueg, K.W., Schuytema, G.S., and Krawczyk, D.F. 1986. Effects of sample storage on a copper-spiked freshwater sediment. Environ. Toxicol. Chem. **5**: 245–253.

Marinsky, J.A., and Ephraim, J.H. 1986. A unified physicochemical description of the protonation and metal ion complexation equilibria of natural organic acids (humic and fulvic acids). 1. Analysis of the influence of polyelectrolyte properties on protonation equilibria in ionic media: fundamental concepts. Environ. Sci. Technol. **20**: 349–354.

Martinez, D.E., and Levinton, J.E. 1996. Adaptation to heavy metals in the aquatic oligochaete *Limnodrilus hoffmeisteri*: evidence for control by one gene. Evolution, **50**: 1339–1343.

Matisoff, G. 1995. Effects of bioturbation on solute and particle transport in sediments. *In* Metal contaminated aquatic sediments. *Edited by* H.E. Allen. Ann Arbor Press, Chelsea, Mich. pp. 201–272.

McGuire, D.L. 1989. Aquatic macroinvertebrate surveys in the Clark Fork River, 1986–1988. Montana Water Quality Bureau, Billings, Mont.

Medine, A.J., Porcella, D.B., and Adams, V.D. 1980. Heavy metal and nutrient effects on sediment oxygen demand in three-phase aquatic microcosms. *In* Microcosms in ecological research. *Edited by* J.P. Giesy. National Technical Information Center, Springfield, Va. pp. 279–303.

Meinelt, T., Kruger, R., Pietrock, M., Osten, R., and Steinberg, C. 1997. Mercury pollution and macrophage centres in pike (*Esox lucius*) tissues. Environ. Sci. Pollut. Res. **4**: 32–36.

Menzie, C., Henning, M.H., Cura, J., Finkelstein, K., Gentile, J., Maughan, J., Mitchell, D., Petron, S., Potocki, B., Svirsky, S., and Tyler, P. 1996. A weight-of-evidence approach for evaluating ecological risks: report of the Massachusetts Weight-of-Evidence Workgroup. Hum. Ecol. Risk Assess. **2**: 277–304.

Miller, M.P., and Hendricks, A.C. 1996. Zinc resistance in *Chironomus riparius*: evidence for physiolotical and genetic components. J. N. Am. Benthol. Soc. **15**: 106–116.

Mills, A.L., and Colwell, R.R. 1977. Microbial effects of metal ions in Chesapeake Bay water and sediment. Bull. Environ. Contam. Toxicol. **18**: 99–103.

Miron, G., and Kristensen, E. 1993. Behavioral response of three nereid polychaetes to injection of hydrogen sulfide inside burrows. Mar. Ecol. Prog. Ser. **101**: 147–155.

Moffett, J.W., Zika, R.G., and Brand, E. 1987. Solvent extraction of copper acetyl-acetonate in studies of copper(II) speciation in seawater. Mar. Chem. **21**: 301–313.

Mohan, R.K. 1996. Technologies for remediating contaminated marine sediments: a state of the art review. Port Technol. Int. **4**: 225–237.

Morel, F.M.M., and Hering, J.G. 1993. Principles and applications of aquatic chemistry. John Wiley & Sons, Inc., New York.

Morse, J.W., Millero, F.J., Cornwell, J.C., and Rickard, D. 1987. The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. Earth Sci. Rev. **24**: 1–42.

Mudroch, A., and Bourbonniere, R.A. 1991. Sediment preservation, processing and storage. *In* CRC handbook of techniques for aquatic sediment sampling. *Edited by* A. Mudroch and S.D. MacKnight. CRC Press, Boca Raton, Fla. pp. 131–169.

Mudroch, A., and MacKnight, S.D. 1991. Bottom sediment sampling. *In* CRC handbook of techniques for aquatic sediment sampling. *Edited by* A. Mudroch and S.D. MacKnight. CRC Press, Boca Raton, Fla. pp. 29–95.

Muller, F.L.L., and Kester, D.R. 1990. Voltammetric determination of the complexation parameters of zinc in marine and estuarine waters. Mar. Chem. **33**: 71–90.

Munawar, J., Munawar, I.F., and Leppard, G.G. 1989. Early warning assays: an overview of toxicity testing with phytoplankton in the North American Great Lakes. Hydrobiologia. **188/189**: 237–246.

Munger, C., and Hare, L. 1997. Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus punctipennis*): implications for predicting Cd bioaccumulation in nature. Environ. Sci. Technol. **31**: 891–895.

Murdoch, M.H., Chapman, P.M., Johns, D.M., and Paine, M.D. 1997. Chronic effects of organochlorine exposure in sediment to the marine polychaete *Neanthes arenaceodentata*. Environ. Toxicol. Chem. **16:** 1494–1503.

Newman, M.C., and Jagoe, C.H. 1994. Ligands and the bioavailability of metals in aquatic environments. *In* Bioavailability: physical, chemical, and biological interactions. *Edited by* J.L. Hamelink, P.F. Landrum, H.L. Bergmann, and W.H. Benson. Lewis Publishers, Boca Raton, Fla. pp. 39–61.

Nirel, P.M.V., and Morel, F.M.M. 1990. Pitfalls of sequential extractions. Water Res. **24**: 1055–1056.

NRC. 1997. Contaminated sediments in ports and waterways: cleanup strategies and technologies. National Academy Press, Washington, D.C.

O'Donnel, J.R., Kaplan, B.M., and Allen, H.E. 1985. Bioavailability of trace metals in natural waters. *In* Aquatic Toxicology and Hazard Assessment: Seventh Symposium. *Edited by* R.D. Cardwell, R. Purdy, and R.C. Bahner. Spec. Tech. Symp. 854, American Society for Testing and Materials, Philadelphia, Pa. pp. 485–501.

Pagenkopf, G.K. 1983. Gill surface interaction model for trace-metal toxicity to fishes: role of complexation, pH and water hardness. Environ. Sci. Technol. **17**: 342–347.

Papelis, C., Hayes, K.F., and Leckie, J.O. 1988. HYDRAQL: a program for the computation of chemical equilibrium composition of aqueous batch systems including surface-complexation modeling of iron adsorption at the oxide/solution interface. Department of Civil Engineering, Standford University, Stanford, Calif.

Paquette, K.E., and Helz, G. 1997. Inorganic speciation of mercury in sulfide waters: the importance of zero-valent sulfur. Environ. Sci. Technol. **31**: 2148–2153.

Pascoe, G.A. 1993. Wetland risk assessment. Environ. Toxicol. Chem. **12**: 2293–2307.

Pascoe, G.A., Blanchet, R.J., Linder, G., Palawski, D., Brumbaugh, W.G., Canfield, T.J., Kemble, N.E., Ingersoll, C.G., Farag, A.,

© 1998 NRC Canada

and DalSolgio, J.A. 1994. Characterization of ecological risks in the Milton Reservoir. Environ. Toxicol. Chem. **13**: 2043–2058.

Pedersen, S.N., Lundebye, A.K., and Depledge, M.H. 1997. Field application of metallothionein and stress protein biomarkers in the shore crab (*Carcinus maenas*) exposed to trace metals. Aquat. Toxicol. **37**: 183–200.

Pederson, F., and Petersen, G.I. 1996. Variability of species sensitivity to complex mixtures. Water Sci. Techol. **33**: 109–119.

Pesch, C.E., and Hoffman, G.L. 1982. Adaptation of the polychaete *Neanthes arenaceodentata* to copper. Mar. Environ. Res. **6**: 307–317.

Pesch, C.E., Hansen, D.J., Boothman, W.S., Berry, W.J., and Mahony, J.D. 1995. The role of acid volatile sulfide and interstitial water metal concentrations in determining bioavailability of cadmium and nickel from contaminated sediments to the marine polychaete *Neanthes arenaceodentata*. Environ. Toxicol. Chem. **4**: 129–141.

Peterson, G.S., Ankley, G.T., and Leonard, E.N. 1996. Effect of bioturbation on metal-sulfide oxidation in surficial freshwater sediments. Environ. Toxicol. Chem. **15**: 2147–2155.

Playle, R.C., Gensemer, R.W., and Dixon, D.G. 1992. Copper accumulation on gills of fathead minnows: influence of water hardness, complexation and pH of the gill microenvironment. Environ. Toxicol. Chem. **11**: 381–391.

Playle, R.C., Dixon, D.G., and Burnison, D.G. 1993*a*. Copper and cadmium binding to fish gills: modification by dissolved organic carbon and synthetic ligands. Can. J. Fish. Aquat. Sci. **50**: 2667–2677.

Playle, R.C., Dixon, D.G., and Burnison, D.G. 1993*b*. Copper and cadmium binding to fish gills: estimates of metal–gill stability constants and modelling of metal accumulation. Can. J. Fish. Aquat. Sci. **50**: 2678–2687.

Power, E.A., and Chapman, P.M. 1992. Assessing sediment quality. *In* Sediment toxicity assessment. *Edited by* G.A. Burton Jr. Lewis Publishers, Chelsea, Mich. pp. 1–18.

Revsbech, N.P., Jorgensen, B.B., Blackburn, T.H., and Cohen, Y. 1983. Microelectrode studies of the photosynthesis and O_2, H_2S, and pH profiles of a microbial mat. Limnol. Oceanogr. **28**: 1062–1074.

Reynoldson, T.B., and Zarull, M.A. 1989. The biological assessment of contaminated sediments — the Detroit River example. Hydrobiologia, **188/189**: 463–476.

Ross, P., Jarry, V., and Sloterdijk, H. 1988. A rapid bioassay using green alga *Selenastrum capricornutum* to screen for toxicity in St. Lawrence River sediment elutriates. *In* Function testing of aquatic biota for estimating hazards of chemicals. *Edited by* J. Cairns, Jr. and J.R. Pratt. Spec. Tech. Publ. 988, American Society for Testing and Materials, Philadelphia, Pa. pp. 68–73.

Roy, R.L., and Campbell, P.G.C. 1997. Decreased toxicity of Al to juvenile Atlantic salmon (*Salmo salar*) in acidic soft water containing natural organic matter: a test of the free-ion model. Environ. Toxicol. Chem. **16**: 1962–1969.

Sasson-Brickson, G., and Burton, G.A., Jr. 1991. In situ and laboratory sediment toxicity testing with *Ceriodaphnia dubia*. Environ. Toxicol. Chem. **10**: 201–207.

Scanferlato, V.S., and Cairns, J., Jr. 1990. Effects of sediment-associated copper on ecological structure and function of aquatic microcosms. Aquat. Toxicol. **149**: 23–34.

Scarano, G., Bramanti, E., and Zirino, A. 1992. Determination of copper complexation in sea water by a ligand competition technique with voltammetric measurement of the labile metal fraction. Anal. Chim. Acta, **264**: 153–162.

Schecher, W.D., and McAvoy, W. 1994. MINEQL$^+$, user's manual. Environmental Research Software, Hallowell, Me.

Shea, D., and MacCrehan, W. 1988. Role of biogenic thiols in the solubility of sulfide minerals. Sci. Total Environ. **73**: 135–141.

Sibley, P.K., Ankley, G.T., Cotter, A.M., and Leonard, E.N. 1996. Predicting chronic toxicity of sediments spiked with zinc: an evaluation of the acid-volatile sulfide (AVS) model using a life-cycle test with the midge *Chironomus tentans*. Environ. Toxicol. Chem. **15**: 2102–2112.

Simpson, S.L., Apte, S.C., and Batley, G.E. 1998. Effect of short-term resuspension events on trace metal speciation in polluted anoxic sediments. Environ. Sci. Technol. **32**: 620–625.

Skalski, C. 1991. Laboratory and in situ sediment toxicity evaluations with early life stages of *Pimephales promelas*. M.S. thesis, Wright State University, Dayton, Ohio.

Slotten, D.G., and Reuter, J.E. 1995. Heavy metals in intact and resuspended sediments of a California reservoir, with emphasis on potential bioavailability of copper and zinc. Mar. Freshwater Res. **46**: 257–265.

Smith, M.E., Lazorchak, J.M., Herrin, L.E., Brewer-Swartz, S., and Thoeny, W.T. 1997. A reformulated, reconstituted water for testing the freshwater amphipod, *Hyalella azteca*. Environ. Toxicol. Chem. **16**: 1229–1233.

Smith, R.W., and Jenne, E.A. 1991. Recalculation, evaluation, and prediction of surface complexation constants for metal adsorption on iron and manganese oxides. Environ. Sci. Technol. **25**: 525–531.

Smith, S.L., MacDonald, D.D., Keenleyside, K.A., and Gaudet, C.L. 1996. The development and implementation of Canadian sediment quality guidelines. *In* Development and progress in sediment quality assessment: rationale, challenges, techniques and strategies. *Edited by* M. Munawar and G. Dave. Ecovision World Monograph Series, SPB Academic Publishing, Amsterdam, The Netherlands. pp. 233–249.

Stackhouse, R.A., and Benson, W.H. 1989. Interactions of humic acid with selected trace metals: influence on bioaccumulation in daphnids. Environ. Toxicol. Chem. **8**: 639–644.

Stemmer, B.L., Burton, G.A., Jr., and Sasson-Brickson, G. 1990. Effect of sediment spatial variance and collection method on cladoceran toxicity and indigenous microbial activity determinations. Environ. Toxicol. Chem. **9**: 1035–1044.

Stephenson, M., and Turner, M.A. 1993. A field study of cadmium dynamics in periphyton and in *Hyalella azteca* (Crustacea: Amphipoda). Water Air Soil Pollut. **68**: 341–361.

Suedel, B.C., and Rodgers, J.H., Jr. 1994*a*. Development of formulated reference sediments for freshwater and estuarine sediment testing. Environ. Toxicol. Chem. **13**: 1163–1175.

Suedel, B.C., and Rodgers, J.H., Jr. 1994*b*. Responses of *Hyalella azteca* and *Chironomus tentans* to particle-size distribution and organic matter content of formulated and natural freshwater sediments. Environ. Toxicol. Chem. **13**: 1639–1648.

Sunda, W.G. 1984. Measurement of manganese, zinc and cadmium complexation in seawater using chelex ion exchange equilibria. Mar. Chem. **14**: 365–378.

Suter, G.W., II. 1996. Overview of the ecological risk assessment framework. *In* Ecological risk assessment of contaminated sediments. *Edited by* C.G. Ingersoll, T. Dillon, and G.R. Biddinger. SETAC Press, Pensacola, Fla. pp. 1–6.

Swartz, R.C., Ditsworth, G.R., Schults, D.W., and Lamberson, J.O. 1985. Sediment toxicity to a marine infaunal amphipod: cadmium and its interaction with sewage sludge. Mar. Environ. Res. **18**: 133–153.

Templeton, D.W., and Della, R.P. 1996. Sediment management — key to assessment and disposal strategies. Port Technol. Int. **4**: 185–190.

© 1998 NRC Canada

Tessier, A., and Campbell, P.G.C. 1987. Partitioning of trace metals in sediments: relationships with bioavailability. Hydrobiologia, **149**: 43–52.

Tessier, A., and Campbell, P.G.C. 1991. Comments on "Pitfalls of sequential extractions". Water Res. **25**: 115–117.

Tessier, A., Campbell, P.G.C., and Bisson, M. 1979. Sequential extraction procedures for the speciation of particulate trace metals. Anal. Chem. **51**: 844–851.

Tessier, A., Campbell, P.G.C., Auclair, J.C., and Bisson, M. 1984. Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area. Can. J. Fish. Aquat. Sci. **41**: 1463–1472.

Tessier, A., Carignan, R., Dubreuil, B., and Rapin, C. 1989. Partitioning of zinc between the water column and the oxic sediments in lakes. Geochim. Cosmochim. Acta, **53**: 1511–1522.

Tessier, A., Couillard, Y., Campbell, P.G.C., and Auclair, J.C. 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. Limnol. Oceanogr. **38**: 1–17.

Tessier, A., Carignan, R., and Belzile, N. 1994. Processes occurring at the sediment–water interface: emphasis on trace metals. *In* Chemical and biological regulation of aquatic systems. *Edited by* J. Buffle and R. De Vitre. Lewis Publishers, Boca Raton, Fla. pp. 139–175.

Tessier, A., Fortin, D., Belzile, N., De Vitre, R.R., and Leppard, G.G. 1996. Metal sorption to diagenetic iron and manganese oxyhydroxides and associated organic matter: narrowing the gap between field and laboratory measurements. Geochim. Cosmochim. Acta, **60**: 387–404.

Tipping, E. 1994. WHAM — a chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. Comp. Geosci. **20**: 973–1023.

Tipping, E., and Hurley, M.A. 1992. A unifying model of cation binding by humic substances. Geochim. Cosmochim. Acta, **56**: 3627–3641.

U.S. EPA. 1990. Assessment and remediation of contaminated sediments (ARCS) work plan. U.S. Environmental Protection Agency, Great Lakes National Program Office, Chicago, Ill.

U.S. EPA. 1992. Framework for ecological risk assessment. EPA/630/R-92/001, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1996. Ecotox thresholds. EPA540/F-95/038, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1997. An SAB report: review of the Agency's draft ecological risk assessment guidelines. EPA-SAB-EPEC-97-002, Science Advisory Board, U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA/ACOE. 1994. Evaluation of dredged material proposed for discharge in waters of the U.S. – testing manual (draft). EPA-823-B-94-002, U.S. Environmental Protection Agency and U.S. Army Corps of Engineers, Washington, D.C.

van den Berg, G.A. 1998. Determination of metal speciation in seawater using cathodic stripping voltammetry. *In* Metals in surface waters. *Edited by* H.E. Allen, W. Garrison, and G.W. Luther. Ann Arbor Press, Chelsea, Mich. pp. 133–153.

van den Berg, G.A., Loch, J.P.G., van der Heijdt, L.M., and Zwolsman, J.J.G. 1998. Vertical distribution of acid-volatile sulfide and simultaneously extracted metals in a recent sedimentation area of the River Meuse in The Netherlands. Environ. Toxicol. Chem. **17**: 758–763.

Vasconcelos, M.T.S.D., Azenha, M.A.O., and Cabral, J.P.S. 1997. Comparison of availability of copper(II) complexes with organic ligands to bacterial cells and to chitin. Environ. Toxicol. Chem. **16**: 2029–2039.

Visscher, P.T., Beukema, J., and Van Gemerden, H. 1991. In situ characterization of sediments: measurements of oxygen and sulfide profiles with a novel combined needle electrode. Limnol. Oceanogr. **36**: 1476–1480.

Walsh, G.E., Weber, D.E., Brashers, L.K., and Simon, T.L. 1990. Artificial sediments for use in tests with wetland plants. Environ. Exp. Bot. **30**: 391–396.

Walsh, G.E., Weber, D.E., Esry, L.K., Nguyen, M.T., Noles, J., and Albrecht, B. 1992. Synthetic substrate for propagation and testing of soil and sediment organisms. Pedobiologia, **36**: 1–20.

Wang, F., Chen, J., and Forsling, W. 1997. Modeling sorption of trace metals on natural sediments by surface complexation model. Environ. Sci. Technol. **31**: 448–453.

Wardlaw, C. 1995. Remediation of metal contaminated sediments: the Canadian experience on the Great Lakes. *In* Remediation of contaminated sediments. *Edited by* P.B. Roeters and G.N.M. Stokman. Hageman Verpakkers, Zoetermeer, The Netherlands. pp. 51–65.

Wentsel, R., McIntosh, A., and Atchison, G. 1978. Evidence of resistance to metals in larvae of the midge *Chironomus tentans* in a metal contaminated lake. Bull. Environ. Contam. Toxicol. **20**: 451–455.

Westrich, J.T., and Berner, R.A. 1984. The role of sedimentary organic matter in bacterial sulfate reduction: the G model tested. Limnol. Oceanogr. **29**: 236–249.

Wildhaber, M.L., and Schmitt, C.J. 1996. Hazard ranking of contaminated sediments based on chemical analysis, laboratory toxicity tests, and benthic community composition: prioritizing sites for remedial action. J. Gt. Lakes Res. **22**: 639–652.

Wood, C.M. 1992. Flux measurements as indices of H^+ and metal effects on freshwater fish. Aquat. Toxicol. **22**: 239–264.

Woodward, D.F., Brumbaugh, W.G., DeLonay, A.J., Little, E.E., and Smith, C.E. 1993. Effects on rainbow trout fry of a metals-contaminated diet of benthic invertebrates from the Clark Fork River, Montana. Trans. Am. Fish. Soc. **123**: 51–62.

Xue, H., and Sigg, L. 1993. Free cupric ion concentration and Cu(II) speciation in a eutrophic lake. Limnol. Oceanogr. **38**: 1200–1213.

Xue, H., and Sigg, L. 1994. Zinc speciation in lakewaters and its determination by ligand-exchange with EDTA and differential pulse anodic stripping voltammetry. Anal. Chim. Acta, **284**: 505–515.

Zhang, J.-Z., and Millero, J. F. 1994. Investigation of metal sulfide complexes in sea water using cathodic stripping square wave voltammetry. Anal. Chim. Acta, **284**: 497–504.

Zhuang, Y., Allen, H.E., and Fu, G. 1994. Effect of aeration of sediment on cadmium binding. Environ. Toxicol. Chem. **13**: 717–724.

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Multiple spawning and the dynamics of fish populations: inferences from an individual-based simulation model

Susan K. Lowerre-Barbieri, James M. Lowerre, and Luiz R. Barbieri

Abstract: We used an individual-based Monte Carlo simulation model to assess how aspects associated with multiple spawning (within a spawning season) affected survivorship, lifetime fecundity, cohort egg production, and yield-per-recruit of a highly exploited species. To make our model more realistic, we included and tested the effects of individual variability in growth and a seasonal growth pattern. Birth months influenced when fish first matured and became vulnerable to the fishery. There was a sixfold increase in mature fish at the beginning of their first spawning season associated with having been born early versus late the previous season. Early born fish had a lower average life-span than later born fish. Although early born fish had lower survivorship they produced the most eggs because of an early size at first maturity, low fishing mortality in the first year, and their larger size at age. These results suggest multiple spawning can have important implications for recruitment and adult population dynamics.

Résumé : Nous avons utilisé un modèle de simulation Monte Carlo fondé sur l'individu pour évaluer comment les aspects connexes au frai multiple (au sein d'une saison de frai) influent sur la survie, la fécondité pendant la durée de vie, la production d'oeufs de la cohorte et le rendement par recrue d'une espèce fortement exploitée. Afin de rendre le modèle plus réaliste, nous avons inclus et testé les effets de la variabilité individuelle de la croissance et d'un régime de croissance saisonnier. Le mois de la naissance influe sur le moment où le poisson devient mature et vulnérable à la pêche. Il y avait une augmentation par un facteur de six des poissons matures au début de leur première saison de frai qui découlait du fait qu'ils étaient nés tôt, comparativement à tard au cours de la saison antérieure. Les poissons nés plus tôt présentaient une durée de vie moyenne inférieure. Bien que les poissons nés plus tôt avaient un taux de survie inférieur, ils produisaient le plus d'oeufs à cause d'une taille à maturité atteinte plus tôt, d'une faible mortalité par pêche au cours de la première année et d'une taille à l'âge plus importante. Ces résultats portent à croire que le frai multiple peut avoir des incidences importantes pour le recrutement et la dynamique de la population adulte.

[Traduit par la Rédaction]

Introduction

Although it has become increasingly recognized that managers need information on how fishing affects reproductive output (Prager et al. 1987; Murphy et al. 1994; Schirripa and Goodyear 1994), traditional models rarely incorporate the dynamic patterns of multiple spawners. This is due in part to the fact that scientists have only recently realized many fish have indeterminate fecundity (Hunter et al. 1985). Prior to this, it was assumed that most fish had determinate fecundity, i.e., annual fecundity can be estimated from the standing stock of advanced oocytes within the ovary before spawning has been initiated (Hunter et al. 1985). In addition, it was assumed that egg production was proportional to spawning biomass. However, more recent work has indicated that numerous species are multiple spawners with indeterminate fecundity (e.g., northern anchovy, *Engraulis mordax* (Hunter and Macewicz 1985); bay anchovy, *Anchoa mitchilli* (Luo and Musick 1991); Atlantic croaker, *Micropogonias undulatus* (Barbieri et al. 1994)), which continuously mature new spawning batches throughout a typically protracted spawning season. For these species, spawning biomass cannot be assumed to be a good indicator of annual reproductive potential (Parrish et al. 1986).

The multiple spawning pattern of these fishes affects their life history in a number of important ways. For example, the extended spawning period of most multiple spawners (regardless of whether they have determinate or indeterminate fecundity), in conjunction with the plasticity of fish growth, insures a large size-at-age range. This large range of sizes at age will affect when fish mature, with individual fish often maturing at different months within a spawning season or even different years (Luo and Musick 1991). It will similarly affect when fish first become vulnerable to capture in a managed fishery with minimum size limits, which may cause differential adult survivorship associated with birth date (i.e., early or late in the spawning season) and hence affect the size and age composition of the breeding population and potentially drive the evolution of the stocks (Law and Grey 1989; Murphy et al. 1994).

Because fecundity of a multiple spawner is determined by

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size at first maturity, batch fecundity, spawning frequency, and seasonal spawning duration, there is potential for a much higher level of variability than with determinate fecundity. Batch fecundity and first maturity are both closely related to size and will vary because of multiple spawners' wide size-at-age range. Batch fecundity-at-size has also been shown to vary individually and for some species to vary between seasons (Hunter et al. 1985; Kjesbu et al. 1996). In addition, spawning frequency and duration have been found to vary within season and between seasons (Kjesbu et al. 1996; Lowerre-Barbieri et al. 1996*b*), as well as by age (Parrish et al. 1986) and individually (Kjesbu et al. 1996).

Traditional yield- and eggs-per-recruit models, although useful, greatly oversimplify how fishing affects multiple spawners. These models combine many individuals into one group (i.e., all fish of a particular age or size), assuming these fish will mature, spawn, grow, and die at the same rates over their life-spans. Although this level of aggregation may produce worthwhile results depending on the questions being asked, ecologists have become aware that individual variability can have a decisive influence on population dynamics and that populations exhibiting substantial individual variation in characteristics important to survival might better be analyzed at a lower level of aggregation (Crowder et al. 1992; Rice et al. 1993).

Based on these considerations, we developed an individual-based Monte Carlo simulation model (IBM) to assess how various aspects associated with multiple spawning affect survivorship, lifetime fecundity, cohort egg production, and yield-per-recruit (YPR). Input data came from a 4-year field study of weakfish, *Cynoscion regalis*, conducted in Chesapeake Bay (Lowerre-Barbieri 1994). Our reasons for choosing an IBM were twofold: (*i*) we were specifically interested in incorporating the individual variability observed in both growth and reproductive parameters of multiple spawners; and (*ii*) the flexibility of this modeling approach made it relatively easy to incorporate a large degree of biological detail in the current model, as well as to augment it in the future. Our overall objective was to build as realistic a model as possible, given the available data. Using a set of baseline parameters, we looked at how fishing and reproduction interacted both at the cohort and individual level (i.e., when fish matured and became vulnerable to the fishery and how birth date affected survivorship and fecundity). We then tested how different aspects of this model affected estimates of yield and egg production. Specifically, we addressed (*i*) the effect of a large range of size at age on vulnerability to the fishery and time of maturity; (*ii*) the effect of individual variability in growth on yield and egg production; (*iii*) the interaction between seasonal growth and an extended spawning season; (*iv*) the effect of differential reproductive output within the spawning season (e.g., more eggs produced at the beginning or end of the season); and (*v*) differential survivorship.

We chose to model weakfish because it is a multiple spawner with indeterminate fecundity and an extended spawning season and because detailed reproductive data were available (Lowerre-Barbieri et al.1996*a*, 1996*b*). Weakfish are most abundant and heavily exploited in waters of the Atlantic from North Carolina to New York. Although weakfish are considered a unit stock, they are highly migratory, overwintering off of North Carolina and in the spring moving northward and inshore to estuarine feeding and spawning grounds. Because of this pattern, they may experience differing fishing pressure depending on season and location, similar to a number of other commercially and recreationally important species along the east coast of the United States.

Methods

Model description

Using a Monte Carlo simulation, individual adult growth, mortality, and egg production of a weakfish year-class were followed on a monthly basis. To keep computing time and the amount of output data reasonable, simulations were based on 4000 fish. Simulations started on January 1 of the first year of life and ran for 25 years (maximum reported age under high exploitation is 17 years; Lowerre-Barbieri et al. 1995). We assumed age-1 fish were made up of four subcohorts, corresponding to birth months, as weakfish spawn from May to August (Lowerre-Barbieri et al. 1996*a*). To focus on the effect of birth date without the potentially confounding effect of differential growth, we assumed all subcohorts exhibited the same growth rate from birth to January 1.

Each subcohort had a different mean size at age 1 (L_1), with earlier born subcohorts being larger than late-born ones. We assumed that the predicted von Bertalanffy TL at age 1 (178 mm based on fish collected in April and May; Lowerre-Barbieri et al. 1995) was a good estimate of mean L_1 on May 1. Because it is difficult to daily age Chesapeake Bay weakfish (Szedlmayer et al. 1991), it was not possible to assign birth dates to these fish. Therefore, we assumed that mean L_1 corresponded to an average birth date, which was assumed to be in the middle of the spawning season (30 June) making them 304 days old. Based on this estimate, we calculated an average daily growth rate of 0.59 mm per day (i.e., 178 mm TL/304 days) and applied it to subcohort-specific ages using a midmonth birth date, resulting in the following L_1s: (*i*) 207 mm TL for May; (*ii*) 188 mm TL for June; (*iii*) 171 mm TL for July; and (*iv*) 152 mm TL for August. Individual L_1s for each subcohort were then simulated based on a random sample from a bivariate normal population with the given means and a coefficient of variation (CV) of 10%. This CV was chosen because it generated what we considered a conservative range of L_1s (100–260 mm TL), based on our field observations (140–255 mm TL; Lowerre-Barbieri et al. 1995) and those reported by Chao and Musick (1977) for April and May (65–330 mm TL; 1977).

Growth was simulated using a seasonally modified von Bertalanffy growth model and compared with that based on the regular von Bertalanffy growth curve. We chose to incorporate this because most temperate water fish demonstrate seasonal growth (Moreau 1987). However, the weakfish migrational pattern makes it difficult to obtain data on seasonal growth (Lowerre-Barbieri et al. 1995). Based on weakfish life history and Chesapeake Bay water temperatures, we modeled growth to be zero in December and maximum in June. Seasonal growth curves were estimated using the following equation:

$$l_t = L_\infty\left(1 - e^{-K(t-t_0)+\frac{6K}{\pi}\left[\sin\left(\frac{\pi}{6}(t-12)\right)-\sin\left(\frac{\pi}{6}(t_0-12)\right)\right]}\right)$$

where l_t is the length at time t, L_∞ is the asymptotic length, K is the Brody growth coefficient, t_0 is the hypothetical time at which length equals zero, t is the time in months.

Three TLs per month were calculated for each fish: at the beginning of the month, the end of the month, and the mean for the month (the average of these two). An estimated annual t_0 of –0.13 and a mean L_∞ of 919 mm TL (Lowerre-Barbieri et al. 1995) were

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used for all fish because there is no data on subcohort-specific growth. The annual t_0 was converted to a monthly t_0 to conform to the model's time frame. Individual L_∞s were simulated with the given mean, a CV of 10%, and a 0.95 correlation between individual L_1s and L_∞s. This generated a reasonable range of L_∞s (550–1250 mm TL; maximum reported TL when highly exploited is 960 mm TL (Villoso 1989) and weakfish have a large size-at-age range). Individual Brody growth coefficients (K) were then generated based on the given t_0, L_1, and L_∞ for each fish.

Whether a fish died in any given month was dependent on its size and the monthly natural mortality (M) and fishing mortality (F) rates. If a fish's TL was <TL at first capture (l_c), then the probability it died was calculated as $1 - e^{-M}$. If its TL was >l_c, then the probability it died was calculated as $1 - e^{-(M+F)}$. For a fish that died, the probability it was caught was calculated as $F/F + M$. Because of a lack of data, M was assumed to be evenly distributed over the year and throughout a cohort's life-span. A monthly M of 0.0167 was used, which corresponds to an annual rate of approximately 0.20 (Lowerre-Barbieri 1994). There are no published estimates of total mortality (Z) or F for weakfish in Chesapeake Bay. The most recently reported is a mean Z of 1.08 for the years 1956–1958 (Vaughan et al. 1991). We chose to use this, although outdated, as it was similar to the recent coastwide annual Z of 1.2, and weakfish are considered a unit stock (Vaughan et al. 1991). Because there are fewer fisheries targeting weakfish during November–March (the months when weakfish are migrating or overwintering off North Carolina), we assumed a lower F. Thus, an annual F of 1.08 was broken down to a monthly F of 0.114 for April–October (the months most weakfish occur in Chesapeake Bay) with a 50% decrease in F (0.057) in November–March.

Size at first capture also differed temporally (i.e., whether it was a month in which weakfish occurred in Chesapeake Bay or not). For April through October, l_c was set at 305 mm TL (12 in.) based on the 1993 minimum size regulations in the Chesapeake Bay region of 305 mm TL in Maryland and 305 mm for recreational and gill nets in Virginia. Based on the landings reported from the North Carolina winter trawl fishery (Ross 1991), we chose a conservative l_c of 200 mm TL for November through March. Recruitment to the fishing gear was assumed to be knife-edged.

Spawning was initiated if a fish was alive during a month within the spawning season (May–August) and the fish was mature. The size-at-first-maturity range was based on field studies in which females <170 mm TL were immature and >210 mm were mature (Lowerre-Barbieri et al. 1996*a*). Thus, within the spawning season the probability a fish was mature was assigned as (*i*) 0% if TL < 170 mm; (*ii*) 50%, if 170 mm < TL < 210 mm; and (*iii*) 100% if TL > 210 mm. Once a fish initiated spawning it continued to spawn. All fish were assumed to be female.

Batch fecundity (BF) was based on an individual fish's mean monthly TL and a field-generated BF to TL relationship (Lowerre-Barbieri et al. 1996*b*). The relationship used was

$$\mathrm{BF} = -349\,558 + 1544\,\mathrm{TL} \qquad R^2 = 0.53, n = 62$$

Although this function was linear for the size range sampled (296–429 mm TL), BF to TL is most likely nonlinear at smaller sizes (e.g., this relationship predicts negative BF for fish <226 mm TL). Thus, a correction factor was used for mature females between 170 mm and 226 mm TL. Batch fecundities for these smaller fish were estimated by multiplying the predicted BF of that fish in the same month at age 3 (age when all fish are big enough to produce a positive BF) by the following ratio:

$$\frac{\mathrm{TL}_{\mathrm{current}} - 170}{\mathrm{TL}_{\mathrm{age3}} - 170}$$

where 170 mm TL is the size at which a fish can first become mature.

Monthly egg production of mature fish was calculated as BF times spawning frequency. Individual monthly spawning frequencies were simulated based on a random sample from a bivariate normal population with a season-long mean spawning frequency of one spawn every 3 days or 10 times per month (Lowerre-Barbieri et al. 1996*b*) and a CV of 10%. Individual annual egg production was calculated as the summation of monthly egg production over the spawning season (May–August).

Model output

The model, based on the above calculations, was adapted to generate two different types of output: (*i*) summary statistics, both annual and for the cohort as a whole, and (*ii*) output for individual fish. For simplicity, we will refer to these as the production version (summary statistics) and the individual version. To make statistical comparisons, the production model was programmed to execute 30 runs using the same input data (i.e., growth parameters, etc.), changing only the initial random number seed. This run size was chosen a priori because a sample size of 30 approximates the normal distribution (Lindgren 1976). Output was saved separately for each run and consisted of egg production, spawning biomass, number of fish that died naturally, number caught, number still alive, and yield for each year the program ran. Yield was calculated as the summation of individual total gutted weights of caught fish. Total gutted weight (TGW) refers to total weight minus the stomach, as weakfish can eat fish up to a third of their own weight (Lowerre-Barbieri et al. 1995). Total length (mm) was converted to TGW (g) using the equation from Lowerre-Barbieri et al. (1995):

$$\mathrm{TGW} = 4.7 \times 10^{-6}\mathrm{TL}^{3.13} \qquad R^2 = 0.99, n = 950$$

Yield per recruit (in grams) was calculated as total yield divided by the number of fish that did not die from natural mortality before reaching a catchable size (200 mm TL).

The individual version was run 10 times for each set of parameters being assessed. This number of runs was chosen to minimize the effects due to random variability, yet keep the amount of output data manageable. The individual version provided output for each fish consisting of: subcohort, status (alive, died naturally, caught), TL, TW, age in months, cumulative egg production, L_1, and L_∞.

Simulations

For baseline data, the models were run with F as outlined above, seasonal growth, equal subcohort sizes, individually variable growth, and fecundity calculated as the product of batch fecundity and spawning frequency (later sections will refer to the use of these parameters as the "baseline model"). These results were compared with those of a virgin stock. To explore the effect of this fishing regime on individuals, size at capture and month of capture were analyzed for each year fish survived. To evaluate the effect of individuals maturing at different times, egg production from the baseline model was compared with that if the model was run with all fish maturing before their first spawning season.

To evaluate the effect of individual variability in growth, the production model was run with subcohort-constant L_1s and L_∞s. This output was compared with that from the baseline parameters. The effect of individual variability in growth on when fish matured and became vulnerable to fishing was also examined by running the individual baseline model with no mortality. To assess when fish matured, the individual model was run for 5 months (beginning of the spawning season) and 8 months (end of the spawning season). To determine when fish became vulnerable to the Chesapeake Bay fishery, the model was run for 10 months (end of the first fishing season), 16 months (beginning of the second season), and 22 months (end of the second season). These results were then compared with those from the individual model when there was no variability in growth.

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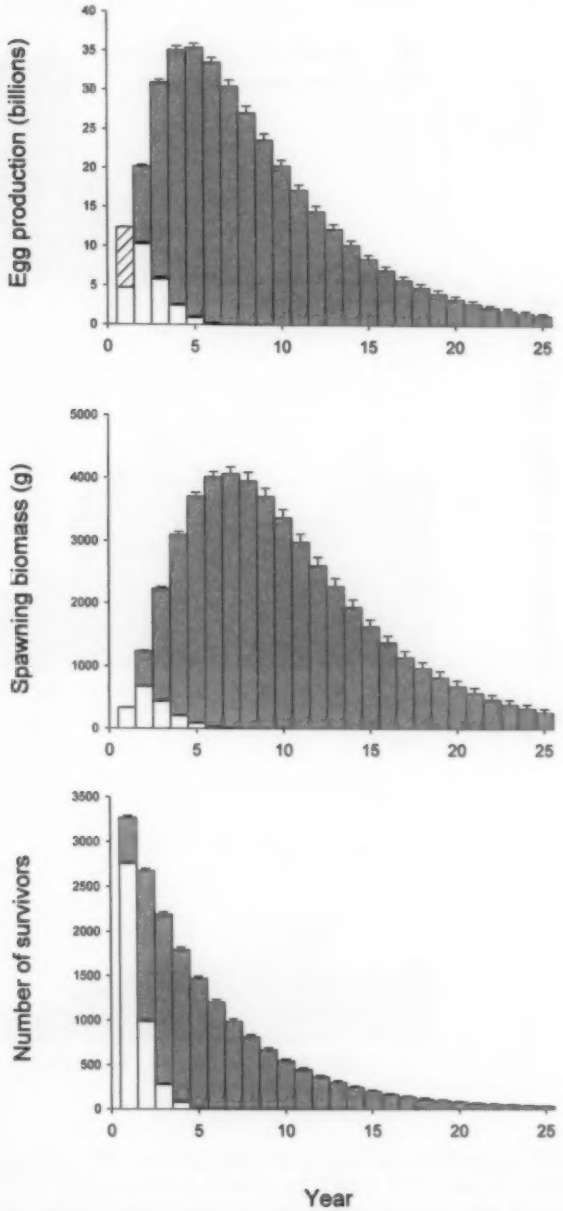
Fig. 1. Annual mean (n = 30 runs) egg production and spawning biomass (±1SD) for a virgin (shaded bar) and fished (open bar) stock. Hatched bar indicates mean egg production in year 1 of a fished stock if all fish matured by the beginning of their first spawning season.



Similarly, to evaluate other effects, specific parameters were changed (as outlined below), while the remainder of the parameters were kept at their baseline values. Seasonal growth was evaluated by running the production model with the regular von Bertalanffy curve. To evaluate the effect of subcohorts corresponding to the extended spawning season of a multiple spawner, the production model was run with all subcohorts having the same mean L_1 of 178 mm, and the output was compared with that of the baseline model. Because spawning activity within the spawning season can vary, with peak egg production sometimes occurring early and in other years late in the season (Lowerre-Barbieri et al. 1996*a*), we assessed three theoretical subcohort regimes: (*i*) equal subcohort sizes (n = 4000); (*ii*) decreasing subcohort sizes with later birth months (n = 3995); and (*iii*) increasing subcohort sizes with later birth months (n = 3995). Decreasing subcohort runs had 50% fewer fish per subcohort for each consecutive birth month (i.e., May, 2130; June, 1065; July, 533; August, 267). Increasing subcohort runs had the opposite. The production model was run for each of these subcohort regimes for both a fished and virgin stock. To look at differential survivorship, the individual model was run for each subcohort regime for a fished stock.

Results

The baseline model

The baseline fishing regime greatly reduced egg production and the ages contributing to the spawning biomass (Fig. 1). Mean cohort egg production for a virgin stock was 358.3×10^9 eggs but varied by as much as 8.9% between runs. Fishing reduced egg production by 93%. In year 1, egg production by a fished stock was similar to that of a virgin stock, although the fished stock had an average of 16% fewer survivors (Fig. 1). The relative contribution this year-class made to cohort egg production, however, was 19 times greater for the fished stock than the virgin. In year 2 the fished stock's egg production peaked (10.3×10^9 eggs, 42% of cohort egg production), whereas virgin egg production was 20.2×10^9 eggs but only 6% of cohort egg production. Virgin egg production did not peak until year 5, while egg production by the fished stock was negligible by year 6 (<1%).

Although most fish were caught in their first 3 years of life, their vulnerability to fishing differed within each of these years (F = 0.057, l_c = 200 mm TL for November–March; F = 0.114, l_c = 305 for April–October) affecting egg production. Few fish were large enough to be caught during their first spawning season (Figs. 2 and 3). Although F decreased in November and December, most fish caught in year 1 (63%) were captured during these months because of the concurrent decrease in l_c (to 200 mm TL). In year 2, many fish were vulnerable in January–March (Fig. 2), effectively decreasing the number of 2-year-old spawners. By November most 2-year-olds were larger than the Chesapeake Bay l_c of 305 mm TL, so when F decreased, so did the number of fish caught (Fig. 3). By year 3, the smaller l_c in North Carolina had little effect, as most fish had grown past 305 mm TL (Fig. 2).

Birth date (subcohort) affected when fish became vulnerable to fishing (Fig. 4) and consequently their average life-span. Only age 1 fish born in May were large enough to be captured by the trawl fishery in North Carolina in January–March (l_c = 200 mm TL). No age 1 fish were large enough to be captured in April and May in Chesapeake Bay (l_c = 305 mm TL). However, May-born fish became vulnerable in June. In contrast, August-born fish were not caught until November and December when l_c again dropped to 200 mm TL. Consequently, the May subcohort had the lowest average life-span of 16.9 months and the August subcohort the longest average life-span of 20.8 months.

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Fig. 2. Total length (TL) and month when individual fish (solid circles) were caught with baseline parameters for years 1–3. Broken lines are size (TL) at first capture of 200 mm during winter months and 305 mm in Chesapeake Bay.

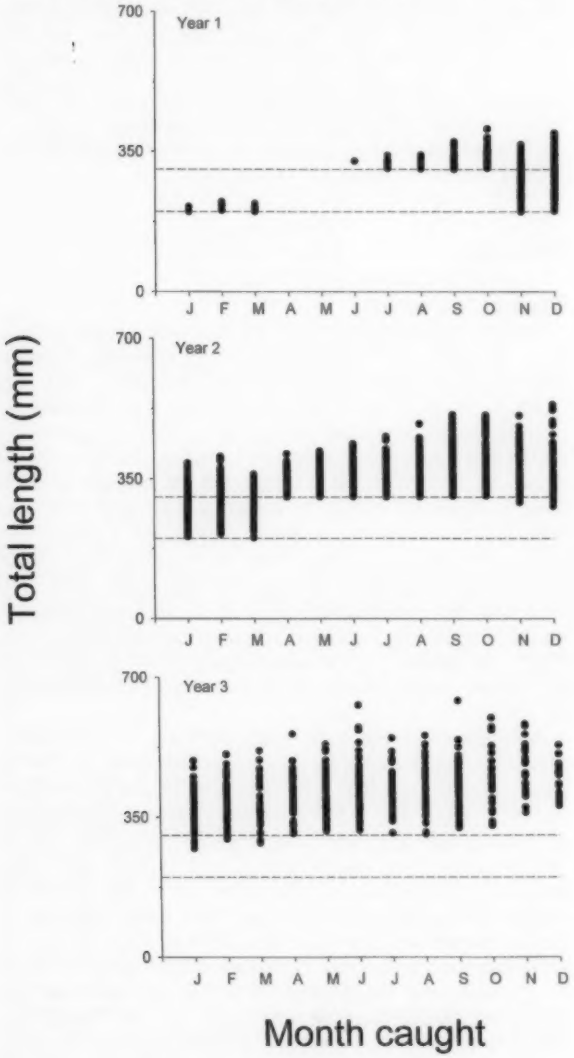
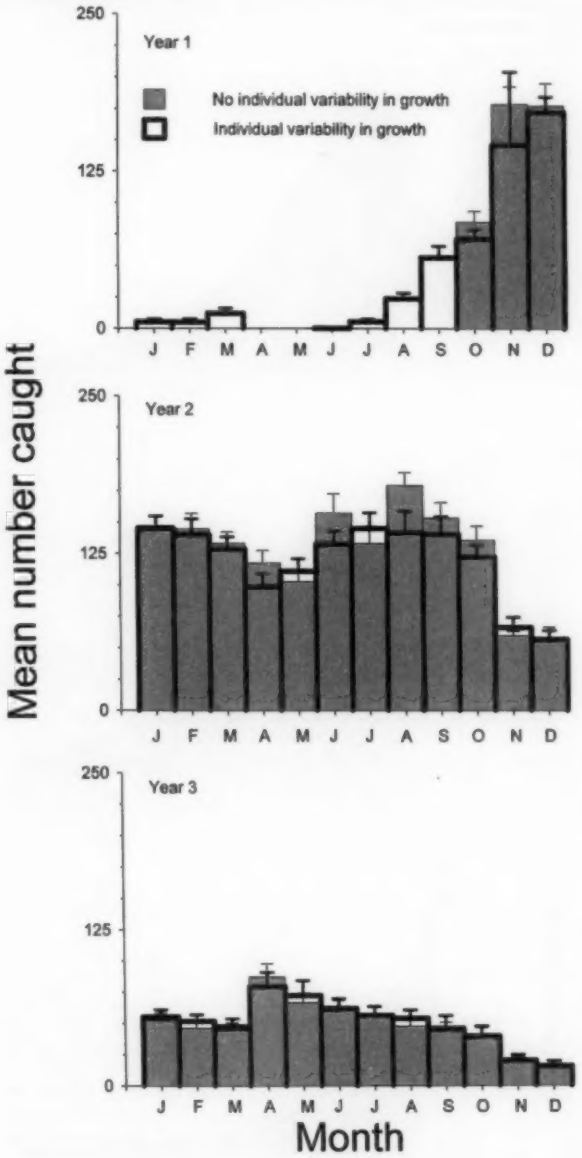


Fig. 3. Mean number of fish caught (n = 30 runs) by month (±1SD) in the first 3 years of life, with and without individual variability in growth.



Birth date also affected when fish matured, with August-born females maturing the slowest. Only 14% of August-born fish were mature by May of their first year compared with 80% of May-born fish. Similarly, all May-born fish matured by the end of the first spawning season but only 87% of August-born fish. Because of this and larger batch fecundities (due to larger body size), early born fish had greater average lifetime fecundities than later born (e.g., May born, 6.9×10^9; August born, 5.1×10^9).

This differential maturity affected which subcohort produced the most eggs and overall cohort egg production. Had all fish been mature by the beginning of their first spawning season, the June and July subcohorts would have had the greatest lifetime fecundities (June, 81.6×10^9; July, 83.2×10^9) compared with early born (May, 80.1×10^9) and late born (August, 76.7×10^9). In addition, peak egg production would have been in year 1 and been more than double that with differential maturity (12.4×10^9 vs. 4.7×10^9 eggs), even though spawning biomass didn't peak until year 2 (Fig. 1).

Effect of individual variability in growth

Modeling all individuals in a subcohort with the same growth pattern resulted in higher egg production and YPR than when growth was modeled as individually variable

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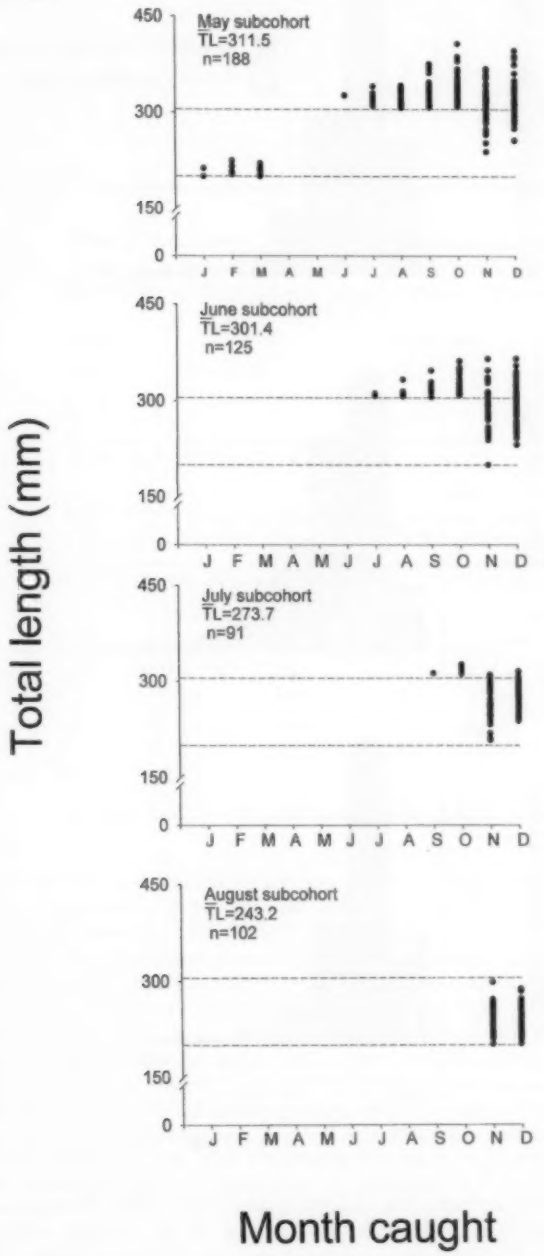
Fig. 4. Total length (TL) and month when individual age 1 fish (each dot) were caught by subcohort. The subcohort regime was equal sized. Broken lines are size (TL) at first capture of 200 mm during winter months and 305 mm in Chesapeake Bay. Number of fish caught and their mean TL are indicated for each subcohort.



(Fig. 5). Mean cohort egg production with subcohort-constant growth was 26.1×10^9, significantly greater than 24.4×10^9 (t-test, $n = 60$, $P < 0.001$) with individually variable growth. Yield per recruit was also significantly greater (t-test, $n = 60$, $P < 0.001$) with nonvariable growth (431.8 g) than when growth varied for individuals (422.3 g).

Although nonvariable growth still resulted in most fish being caught in the first 3 years of life and yield and egg production peaking in year 2, vulnerability to fishing differed somewhat. In year 1, nonvariable growth led to fewer fish being caught (Fig. 3) and a slightly higher egg production than with variable growth. This is primarily because with nonvariable growth fish did not become vulnerable to the fishery until October of their first year, while with variable growth some fish were vulnerable as early as January (Fig. 3). In addition, fish matured slightly faster with nonvariable growth, with 100% mature by the end of the first spawning season with nonvariable growth and 97% with variable growth. Catch rates continued to differ somewhat throughout year 2, with overall vulnerability being slightly greater with nonvariable growth, but by year 3, there was little difference (Fig. 3).

Seasonal growth model

When growth was modeled as seasonal, it resulted in higher egg production and YPR than nonseasonal growth. Mean egg production for a fished stock was significantly greater (t-test, $n = 60$, $P < 0.001$) when growth was modeled as seasonal (24.4×10^9) versus nonseasonal (23.5×10^9). Virtually all of the increased egg production occurred in year 1 because fish mature earlier with seasonal growth. By year 3, annual egg production became less for seasonal growth than nonseasonal growth. Similarly, YPR was significantly greater (t-test, $n = 60$, $P < 0.001$) when growth was modeled as seasonal (422.3) than nonseasonal (415.8) because of a larger mean size of fish caught in year 1 ($\overline{TL}$ = 288.4 mm seasonal; 261.9 mm nonseasonal) and year 2 ($\overline{TL}$ = 388.4 mm seasonal; 327.0 mm nonseasonal).

Effect of birth date on survivorship

Fish with smaller L_1s showed greater survivorship. In 10 runs with equal-size subcohorts, the mean number of survivors ≥ age 3 increased with later birth dates: 34.1, 43.2, 45.3, and 70.0 for the May, June, July, and August subcohorts, respectively. August subcohort survivorship was significantly greater than any other subcohort (ANOVA, $n = 40$, $P < 0.001$; Tukey's test, $P < 0.05$). This increased survivorship was due to the later birth date's smaller L_1, with mean L_1 significantly smaller (ANOVA, $n = 20$, $P < 0.001$) for all fish surviving to ages ≥3 (169.0 mm) than those which died (179.1 mm). Mean number of survivors ≥ age 3 was greater for the increasing subcohort regime (225), intermediate for the equal subcohort regime (193), and lowest for the decreasing regime (164). This was driven by the initial number of fish in the August subcohort (increasing subcohort regime, 2130; equal subcohort regime, 1000; decreasing subcohort regime, 267), which had a smaller mean L_1.

Effect of subcohorts

Modeling weakfish with four equal-sized subcohorts corresponding to birth months had a minimal effect on YPR and egg production. There was a wider L_1 distribution with subcohorts than without, which led to a slight but nonsignificant increase in egg production (24.4×10^9 vs. 24.6×10^9 eggs, t-test, $n = 60$, $P = 0.402$) and a slight but

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significant decrease in YPR (422.3 vs. 417.0 g, *t*-test, n = 60, P = 0.013).

How fish were apportioned to the subcohorts, however, affected egg production and YPR. YPR differed significantly by subcohort regime (ANOVA, n = 90, P = 0.0001). It was largest (466.9 g) when subcohort size was decreasing (most fish in May subcohort), intermediate for equal subcohorts (422.3 g), and smallest (377.2 g) for increasing subcohort size (most fish in August subcohort). Counterintuitively, given that weakfish growth becomes asymptotic at age 6, the greatest YPR did not occur with the subcohort regime producing the oldest survivors (increasing regime) because of its smaller number and size of fish caught in years 1 and 2 (Table 1).

Egg production also differed significantly by subcohort regime (ANOVA, n = 90, P < 0.001), with the decreasing subcohort regime producing the most eggs (Table 2). However, annual egg production was greater for the decreasing subcohort regime only in the first 2 years (Table 3). By year 3, egg production was similar for all three regimes, although somewhat larger for the increasing subcohort regime, which continued to be greater from this point on even though its spawning biomass did not become greater until year 6.

Discussion

The baseline model

Our model incorporated aspects of multiple spawning and demonstrated how they can affect adult population dynamics. An obvious factor, which has been all but ignored, is the effect of a large range of birth dates from an extended spawning season (Cargnelli and Gross 1996). By simulating subcohorts corresponding to birth months with a 10% CV in individual growth we produced a realistic range of sizes at age 1. The differences in body size of subcohorts had far-reaching effects, including when fish matured and became vulnerable to the fishery, the size of cohort egg production and yield, and adult life-span.

There is concern that fisheries using a minimum size limit may act as a new selection pressure, causing earlier maturity and slower growth of various species (Sutherland 1990). Our simulations indicated the larger average size of early born (May) versus late-born (August) subcohorts caused early born fish to mature and become vulnerable to fishing sooner and to have lower survivorship. Thus, selection would be expected to favor spawning later in the season (creating more August-born fish with their smaller size at age). However, greater lifetime survivorship does not necessarily translate into the most eggs. Under the baseline fishing regime the May subcohort had the greatest lifetime fecundity, because of the relatively low fishing mortality during the first year's spawning season, allowing May fish to produce more eggs, because they matured earlier and had a larger size.

We based our subcohort mean size at age 1 on the assumption that the duration of the first growth season was the predominant factor affecting size at age 1. We did this because of a lack of data and because weakfish spawn primarily in the summer, when temperatures are warm and growth is relatively fast. This is similar to the bluegill sunfish, *Lepomis macrochirus*, which spawn primarily in June and July and showed a clear relationship between birth date and yearling body size (Cargnelli and Gross 1996). However, a fall spawning season such as that of the Atlantic croaker might greatly decrease the importance of duration of the first growth season.

Because of a lack of data, we had to base important parameters such as subcohort-specific L_1s, time of peak seasonal growth and monthly F on general assumptions and historical data. To fully assess the impact of these assump-

Fig. 5. Annual mean (n = 30 runs) egg production, yield, and number of fish caught (±1SD) under the baseline fishing regime, with equal-sized subcohorts and seasonal growth, with and without individual variability in growth.

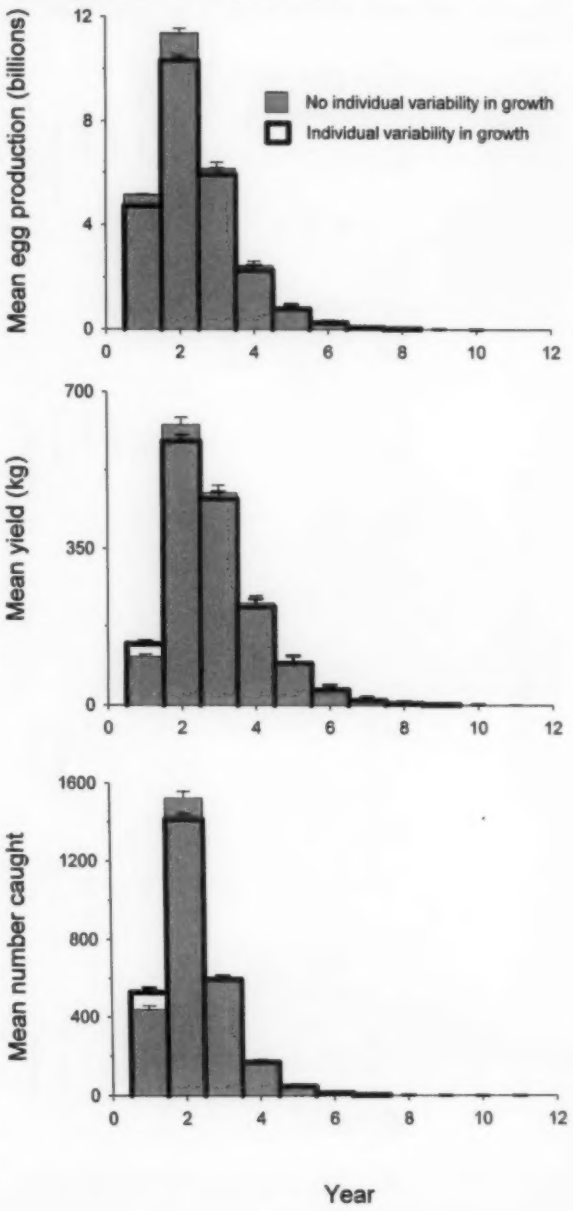

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Table 1. Mean (n = 30 runs) yield, number and average weight (g) of fish caught by year, when subcohorts were modeled as equal, increasing, and decreasing.

	Equal subcohort size			Decreasing subcohort size			Increasing subcohort size		
Year	Yield (kg)	No. caught	Average weight	Yield (kg)	No. caught	Average weight	Yield (kg)	No. caught	Average weight
1	136	528	257	193	657	294	84	410	204
2	589	1415	417	702	1475	476	475	1335	356
3	460	595	774	471	504	936	439	686	639
4	219	168	1302	226	143	1580	218	198	1099
5	93	47	1963	93	41	2259	93	56	1648
6	34	13	2661	34	11	3040	35	15	2294
Total caught	2766			2831			2700		

Table 2. Mean, range, and standard error of egg production (n = 30 runs) for a virgin and fished stock of weakfish, *Cynoscion regalis*, when subcohort size was modeled as equal, increasing and decreasing.

	Equal subcohort size		Decreasing subcohort size		Increasing subcohort size	
Statistic	Virgin	Fished	Virgin	Fished	Virgin	Fished
Mean	358	24	383	26	333	22
Range	346–379	23–26	369–403	25–28	348–319	22–24
SE	1.78	0.11	1.61	0.13	1.43	0.10

Note: Egg production is in billions.

Table 3. Mean (n = 30 runs) spawning biomass (in kg) and egg production (in billions) of a fished stock by year, when subcohorts were modeled as equal, increasing, and decreasing.

	Equal subcohort size			Decreasing subcohort size			Increasing subcohort size		
Year	Spawning biomass	No. alive	Eggs	Spawning biomass	No. alive	Eggs	Spawning biomass	No. alive	Eggs
1	330.8	2757	4.7	443.4	2634	6.1	218.0	2865	3.4
2	670.5	983	10.3	729.5	835	11.2	608.1	1147	9.3
3	434.6	276	5.9	451.0	237	5.8	418.6	327	6.1
4	201.7	77	2.3	211.0	66	2.2	203.6	91	2.5
5	84.1	21	0.8	85.4	19	0.8	83.0	25	0.9
6	29.4	6	0.3	29.6	5	0.2	30.9	7	0.3

tions we will need to do further sensitivity analysis. However, important aspects of our simulation results were similar to those from the field. As already mentioned, simulated L_1 and maximum size ranges were reasonable, given what has been reported for the field. In addition, maximum age in our simulations was age 8, the same as that from general Chesapeake Bay commercial samples from 1989 to 1992 (Lowerre-Barbieri et al. 1995). Our simulations indicated ages 2 and 3 contributed the most to egg production as did our field studies (Lowerre-Barbieri et al. 1996*b*). Also the simulated differential maturity closely followed that reported from the field, with early born fish maturing earlier the following year than later born (Lowerre-Barbieri et al. 1996*b*).

Individual variability in growth

In fishes, important life-history parameters such as survivorship, age-at-maturity, and fecundity are more closely linked to size than age (Kirkpatrick 1984), and many fish demonstrate a large range of sizes at age. Yet traditional fishery models are highly aggregated, assuming all fish of a given age are the same size. By decreasing our level of aggregation, first to subcohorts and then to the individual, fish matured and became vulnerable to the fishery in a more realistic fashion. Thus, larger fish (due to birth date and individual growth differences) matured earlier and on average produced more eggs and died earlier, significantly affecting both yield and egg production. Had all fish been modeled to mature at the beginning of the first spawning season, egg production would have peaked in year 1 rather than year 2. In addition, earlier born fish would not have produced the most eggs, counteracting their lower survivorship. Instead, the July subcohort would have had the greatest lifetime egg production.

Growth model

Although most temperate water fish demonstrate seasonal growth (Moreau 1987), traditional age-based models rarely incorporate it. Our simulations, however, indicate it can have a significant impact on egg production estimates. Peak seasonal growth in June corresponded with the middle of the weakfish spawning season and thus increased egg produc-

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tion. However, this would differ for other species depending on how the time of peak growth corresponded to their spawning seasons. With the fall-spawning Atlantic croaker, one would expect peak growth in June to have either little effect or to lower egg production estimates compared with estimates based on the traditional von Bertalanffy growth model.

We assumed growth peaked in June based on a trade-off between water temperature and spawning energetics. Water temperature at our Chesapeake Bay sampling site peaked in July at the same time that weakfish condition was at its lowest (Lowerre-Barbieri et al. 1996*b*), presumably because of the demands of multiple spawning. Because of the complicated weakfish migrational pattern, better defining weakfish seasonal growth from field data is virtually impossible, although it could be studied in captivity.

Subcohorts and survivorship

Ages 1 and 2 drove subcohort egg production and YPR results. Intuitively, given that the species we modeled does not reach asymptotic growth until age 6, it would seem the regime with the greatest survivorship would have the greatest YPR and egg production. However, this was not the case. In the first 2 years when numbers of fish were relatively high, the larger size of fish in the decreasing subcohort regime (more early born fish) had a greater effect on egg production and YPR than the larger number of fish surviving to older ages with the increasing subcohort regime. Obviously, these results depend on a number of factors such as size at maturity, minimum size limit, age at which growth becomes asymptotic, and mortality rate. However, they do indicate that potential differences in reproductive output over an extended season can affect adult population dynamics. They also underline the importance of young fish to egg production and yield for fish heavily exploited and with a life history similar to that of weakfish.

Summary

It has become increasingly clear that it is essential to model not only the effects of fishing on yield but also on the reproductive capacity of a stock (Norris 1991; Schirripa and Goodyear 1994). However, little attention has been paid to how a multiple-spawning strategy might affect population dynamics. A multiple spawner typically produces eggs over an extended period, while total spawners produce all their eggs at one time within a spawning season. The most obvious advantage for multiple spawners is that their fecundity is not restricted by body cavity size, and thus, they can greatly increase their egg production (Garrod and Horwood 1984). Because of this increased egg production, the number of individuals will have a greater effect on egg production than spawning biomass, as was demonstrated in our simulations by the fact that egg production peaked in year 1 when all fish matured before their first spawning season, even though spawning biomass continued to peak in year 2 (Fig. 1).

Because multiple spawners typically have extended spawning seasons, they also produce a large range of sizes at age, which affects vulnerability to fishing and time of maturity. In our simulations, individuals maturing at different times (differential maturity) had a greater affect on egg production than survivorship, with early born fish producing the most eggs. Thus, assuming more eggs translates into more fish, an increased number of early born fish surviving to their first year would in turn produce more early born fish the next generation and so on. In this fashion, weakfish could go into a cycle of increased abundance without any obvious cause such as a decrease in fishing pressure. However, anything increasing the survival of August-born fish over earlier born would have the opposite effect. Weakfish have gone through such cycles in abundance (Lowerre-Barbieri et al. 1995), and the timing of peak larval abundance has been reported to vary in different years (Lowerre-Barbieri et al. 1996*b*). The effect of differential maturity would be particularly noticeable in a highly exploited fishery like weakfish because the spawning population has been so severely restricted, increasing the importance of the first year's egg production.

These results would not have been apparent with a more highly aggregated, annual model. A monthly time step was necessary to evaluate differential maturity and recruitment to the fishery. Whereas, the individual-based approach allowed us to incorporate a large amount of detailed biological data, which the mathematics of more traditional models would have made extremely tedious. It also allowed us to compare the phenotypic traits associated with fish that were caught versus survivors (e.g., birth month, size at age 1).

Our simulations also have important management implications. The monthly time step made it easy to evaluate the effects of fishing during and outside of the spawning season. It makes it equally easy to evaluate a closed season. However, the differential maturity results indicate that the importance of protecting egg production at different points within the spawning season may vary. Our model also allowed the simulation of different fishing regimes associated with a migratory species encountering different state regulations. Although efforts are being made to standardize regulations, and in fact the North Carolina weakfish minimum size limit has recently been raised to 305 mm TL, many species continue to be exploited at different rates associated with seasonal migrations.

The model we present here also has many limitations. One of these was the same detailed input and output. This level of information is often not available. For example, with weakfish we had to estimate several important parameters based on general assumptions, as well as model theoretical subcohort regimes rather than those observed from the field. In addition, the detailed reproductive estimates such as spawning frequency and batch fecundity, are not available for many species. The current version of this model also demonstrates several of the limitations of more traditional models, such as the assumption of steady state and the estimation of an unfished cohort's biomass without incorporating compensatory mechanisms. It also does not incorporate potential changes in M associated with seasonality or age.

With any IBM there is the problem of how many individuals to use in simulations. In the present model, we were concerned with how a much larger number of fish might change the percentage of fish surviving to older ages and thus the cohort dynamics. However, differences were minimal when we compared the annual number of survivors from a deterministic version of this model run with 400 000 fish versus the IBM version with equal subcohorts and 4000

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fish. The IBM on average (30 runs) had one fish survive to age 8, whereas the deterministic model had two fish survive to age 9. Annual percent survivors was actually a little higher for the IBM because all fish in a subcohort did not become vulnerable to fishing at the same time.

This model is a very simplistic representation of multiple spawning. As shown for the northern anchovy, egg production of a multiple spawner can vary in relation to food, growth, temperature, population size, and age structure. Our simulations looked at the effect of growth, population size, and age structure. Annual temperature fluctuations can be expected to affect when the spawning season begins, growth (and thus batch fecundities), and spawning frequencies. However, its effects can also be confounded by other factors affecting fish condition such as food availability. Unfortunately, studies addressing how these factors affect weakfish spawning have not yet been conducted, but we hope in the future to incorporate temperature and energetics to assess how much egg production can vary from year to year, even if population size remains constant.

Despite these limitations, our results show that IBMs can help improve our understanding of how fishing and multiple spawning interact to affect adult population dynamics. Our simulations provided insight on how various seasonal parameters can cause nonintuitive results, as well as on selective pressures of a size-based fishery on a multiple spawner with an extended spawning season. It did not, however, incorporate aspects of early life history, and there may be a number of early life history traits associated with birth date and spawning energetics that have opposite selective processes, counteracting those of the adult stages. Thus, to fully understand the factors affecting recruitment of multiple spawners it will be necessary to combine detailed information on spawning dynamics and early life history.

Acknowledgments

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References

Barbieri, L.R., Chittenden, M.E., Jr., and Lowerre-Barbieri, S.K. 1994. Maturity, spawning and ovarian cycle of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent waters. Fish. Bull. U.S. **92**: 671–685.

Cargnelli, L.M., and Gross, M.R. 1996. The temporal dimension in fish recruitment: birth date, body size, and size-dependent survival in a sunfish (bluegill: *Lepomis macrochirus*). Can. J. Fish. Aquat. Sci. **53**: 360–367.

Chao, L.N., and Musick, J.A. 1977. Life history, feeding habits, and functional morphology of juvenile sciaenid fishes in the York River estuary, Virginia. Fish. Bull. U.S. **75**: 657–702.

Crowder, L.B., Rice, J.A., Miller, T.J., and Marschall, E.A. 1992. Empirical and theoretical approaches to size-based interactions and recruitment variability in fishes. *In* Individual-based models and approaches in ecology: populations, communities and ecosystems. *Edited by* D.L. DeAngelis and L.J. Gross. Routledge, Chapman & Hall, New York. pp. 237–255.

Garrod, D.J., and Horwood, J.W. 1984. Reproductive strategies and the response to exploitation. *In* Fish reproduction: strategies and tactics. *Edited by* G.W. Potts and R.J. Wooten. Academic Press, New York. pp 367–384.

Hunter, R.H., and Macewicz, B.J. 1985. Rates of atresia in the ovary of the captive and wild northern anchovy, *Engraulis mordax*. Fish. Bull. U.S. **83**: 119–136.

Hunter, R.H., Lo, N.C.H., and Leong, R.H.H. 1985. Batch fecundity in multiple spawning fishes. *In* An egg production method for estimating spawning biomass of pelagic fishes: application to the northern anchovy, *Engraulis mordax*. *Edited by* R. Lasker. NOAA Tech. Rep. No. NMFS 36. pp. 79–94.

Kirkpatrick, M. 1984. Demographic models based on size, not age, for organisms with indeterminate growth. Ecology, **65**: 1874–1884.

Kjesbu, O.S., Solemdal, P., Bratland, P., and Fonn. M. 1996. Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **53**: 610–620.

Law, R., and Grey, D.R. 1989. Evolution of yields from populations with age-specific cropping. Evol. Ecol. **3**: 343-359.

Lindgren, B.W. 1976. Statistical theory. 3rd ed. McMillan, New York.

Lowerre-Barbieri, S.K. 1994. Life history and fisheries ecology of weakfish, *Cynoscion regalis*, in the Chesapeake Bay region. Ph.D. thesis, College of William and Mary, Virginia Institute of Marine Science, Gloucester Point.

Lowerre-Barbieri, S.K., Chittenden, M.E., Jr., and Barbieri, L.R. 1995. Age and growth of weakfish, *Cynoscion regalis*, in the Chesapeake Bay region, with a discussion of historic fluctuations in maximum size. Fish. Bull. U.S. **93**: 642–655.

Lowerre-Barbieri, S.K., Chittenden, M.E., Jr., and Barbieri, L.R. 1996*a*. The multiple spawning pattern of weakfish (*Cynoscion regalis*) in the Chesapeake Bay and Middle Atlantic Bight. J. Fish. Biol. **48**: 1139–1163.

Lowerre-Barbieri, S.K., Chittenden, M.E., Jr., and Barbieri, L.R. 1996*b*. Variable spawning activity and annual fecundity of weakfish in Chesapeake Bay. Trans. Am. Fish. Soc. **125**: 532–545.

Luo, J., and Musick, J.A. 1991. Reproductive biology of the bay anchovy in Chesapeake Bay. Trans. Am. Fish. Soc. **120**: 701–710.

Moreau, J. 1987. Mathematical and biological expression of growth in fishes: recent trends and further developments. *In* Age and growth of fish. *Edited by* R.C. Summerfelt and G.E. Hall. Iowa State University Press, Ames. pp. 15–42.

Murphy, E.J., Rodhouse, P.G., and Nolan, C.P. 1994. Modeling the selective effects of fishing on reproductive potential and population structure of squid. ICES J. Mar. Sci. **51**: 299–313.

Norris, J.G. 1991. Further perspectives on yield per recruit analysis and biological reference points. Can. J. Fish. Aquat. Sci. **48**: 2533–2542.

Parrish, R.H., Mallicoate, D.L., and Klingbeil, R.A. 1986. Age dependent fecundity, number of spawnings per year, sex ratio, and maturation stages in northern anchovy, *Engraulis mordax*. Fish. Bull. U.S. **84**: 503–517.

Prager, M.H., O'Brien, J.F., and Saila, S.B. 1987. Using lifetime fecundity to compare management strategies: a case history for striped bass. N. Am. J. Fish. Manage. **7**: 403–409.

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Rice, J.A., Miller, T.J., Rose, K.A., Crowder, L.B., Marschall, E.A., Trebitz, A.S., and DeAngelis, D.L. 1993. Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. Can. J. Fish. Aquat. Sci. **50**: 133–141.

Ross, J.L. 1991. Assessment of the North Carolina winter trawl fishery September 1985–1988. Spec. Sci. Rep. No. 54. N.C. Department of Environment, Health, and Natural Resources, Division of Marine Fisheries, Morehead City.

Schirripa, M.J., and Goodyear, C.P. 1994. Simulation modeling of conservation standards for spotted seatrout (*Cynoscion nebulosus*) in Everglades National Park. Bull. Mar. Sci. **54**: 1019–1035.

Sutherland, W.J. 1990. Evolution and fisheries. Nature (London), **344**: 814–815.

Szedlmayer, S.T., Able, K.W., Musick, J.A., and Weinstein, M.P. 1991. Are scale circuli deposited daily in juvenile weakfish, *Cynoscion regalis*? Environ. Biol. Fishes, **31**: 87–94.

Vaughan, D.S., Seagraves, R.J., and West, K. 1991. An assessment of the status of the Atlantic weakfish stock, 1982–1988. Special Report. Atlantic States Marine Fisheries Commission, Washington, D.C.

Villoso, E.P. 1989. Reproductive biology and environmental control of spawning cycle of weakfish, *Cynoscion regalis* (Block and Schneider), in Delaware Bay. Ph. D. dissertation, University of Delaware, Newark, N.J.

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Relationships between Alaskan salmon catch and North Pacific climate on interannual and interdecadal time scales

Mary W. Downton and Kathleen A. Miller

Abstract: Using multivariate time series models, Alaska's statewide commercial catch of three salmon species during 1925–1994 is shown to be related to surface temperatures in particular large regions of the eastern North Pacific. Previous research has indicated that interdecadal changes in Alaskan catch levels are related to large-scale changes in the climate regime of the North Pacific. The present work focuses on interannual variability by controlling for climatic shifts in the mid-1940s and mid-1970s. For sockeye (*Oncorhynchus nerka*) and chum (*O. keta*), relationships with temperature occur within a few months after ocean entry with warmer regional temperatures enhancing survival. For sockeye and pink salmon (*O. gorbuscha*), relationships with temperature occur at the time and location of the return migration, suggesting that colder ocean temperatures may enhance spawning success or egg survival. In addition, the models show a significant positive relationship of chum catch to pink catch 2 years earlier, suggesting a common influence on smolts of the two species. The results support the contention that climatic factors affecting the marine environment play a significant role in salmon production on interannual, as well as interdecadal, time scales.

Résumé : À l'aide de modèles de séries chronologiques multicritères, on a montré l'existence d'un lien entre les prises commerciales de l'ensemble de l'Alaska de 1925 à 1994 et les températures en surface dans certaines grandes régions de l'est du Pacifique nord. Des recherches antérieures avaient indiqué que les changements d'une décennie à l'autre dans les niveaux des prises de l'Alaska étaient reliés à des changements à grande échelle dans le régime climatique du Pacifique nord. Les études actuelles, qui portent principalement sur la variabilité d'une année à l'autre, recherchent les changements dans les conditions climatiques vers le milieu des années 40 et vers le milieu des années 70. Dans le cas du saumon rouge (*Oncorhynchus nerka*) et du saumon kéta (*O. keta*), on note l'existence d'un lien avec la température quelques mois après leur arrivée dans l'océan, les températures élevées augmentant le taux de survie. Dans le cas du saumon rouge et du saumon rose (*O. gorbuscha*), un lien avec la température est observé au moment et au site de la migration de retour, ce qui permet de croire que les basses températures océaniques améliorent les chances de succès de la fraye ou de survie des oeufs. De plus, les modèles mettaient en évidence un rapport positif significatif entre les prises de saumon kéta et celles du saumon rose deux ans auparavant, ce qui semble indiquer un effet commun sur les smolts de ces deux espèces. Les résultats obtenus appuient les conclusions selon lesquelles les facteurs climatiques touchant le milieu marin jouent un rôle important dans la production de saumon, tant d'une année à l'autre que d'une décennie à l'autre.

[Traduit par la Rédaction]

Introduction

Production of Alaskan salmon has fluctuated greatly during the 20th century, highlighted by a dramatic increase that began in the late 1970s. Recent research suggests a relationship between shifts in salmon abundance and changes in the climate and marine environment of the North Pacific Ocean (Beamish and Bouillon 1993; Francis and Hare 1994; Brodeur and Ware 1995; Adkison et al. 1996; Mantua et al. 1997). Hare and Francis (1995) show statistically significant step changes in catch of Alaskan sockeye (*Oncorhynchus nerka*) and pink salmon (*O. gorbuscha*) stocks in the late 1940s and late 1970s, which they characterize as alternating low- and high-production regimes. They relate these changes to shifts in mean levels of sea level pressure (SLP) and temperature in the mid-1940s and mid-1970s. Although evidence was found for a relationship of salmon production with both sea level pressure and sea surface temperature (SST) on the interdecadal time scale, no relationship was found at the scale of interannual variability (Hare 1996).

In this study, both interannual and interdecadal variability in Alaskan salmon catch are explored further, in relationship to SST. Statistical time series analysis techniques are used to model annual commercial catch of the three major Alaskan salmon species: sockeye, pink, and chum (*O. keta*).

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Climate variability over the North Pacific

Several features of atmosphere–ocean dynamics influence weather and climate patterns over large areas of the North Pacific and North America. Best known is the El Niño –

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Southern Oscillation (ENSO) phenomenon, which involves alternate warming and cooling of SSTs in a band along the equator from the mid-Pacific to the west coast of South America on a time scale of 3–6 years. Its effects propagate northward leading, in an ENSO warm event, to warming along the west coast of North America and cooling of SSTs in the central North Pacific. During 1976–1998, Pacific climate has been influenced by an unusual sequence of ENSO warm events (Trenberth and Hurrell 1994; Trenberth and Hoar 1996).

Recent research suggests that there is a second mode of variability in North Pacific SST, which is at least partially independent of ENSO (Zhang et al. 1996). Centered in the Northwest Pacific, it is characterized by the warming and cooling, on multidecadal time scales, of SSTs between roughly 35 and 50°N, extending across the entire Pacific Basin. Using a 94-year record of global and regional SSTs, Zhang et al. (1997) show opposite shifts in SST in 1977 and about 1942–1943. The multidecadal climate variability in the central North Pacific has been called the Pacific Interdecadal Oscillation, or PDO (Mantua et al. 1997). As an index of the PDO, Mantua et al. use the first principal component of SSTs for the entire North Pacific poleward of 20°N. It is only weakly correlated with indices of ENSO.

Although ENSO and the PDO characteristically fluctuate on different time scales, they have similar effects in the northeast Pacific and Alaska, particularly evident during the northern winter. The "warm" phase of ENSO in tropical waters and the "positive" phase of the PDO in the extratropics both tend to be associated with intensification of the Aleutian low pressure system, strengthening of westerly winds across the North Pacific, cooling of SSTs in the central North Pacific, and warming of sea and air temperatures along the west coast of North America (Zhang et al. 1996).

A particularly abrupt shift in the North Pacific climate began in the winter of 1976–1977 (Miller et al. 1994). This shift has been attributed by some to the unusual sequence of tropical ENSO events (Trenberth and Hurrell 1994) and by others to the PDO (Mantua et al. 1997). Many changes in the biota of the North Pacific after 1977 have been documented (Venrick et al. 1987; Ebbesmeyer et al. 1990). In the Gulf of Alaska, Brodeur and Ware (1992) show a doubling of the summer zooplankton biomass between the periods 1956–1962 and 1980–1989. The biggest increases occurred along the northern periphery of the Gulf of Alaska, which would most benefit subarctic species, particularly sockeye, pink, and chum salmon.

Implications for Pacific salmon

Salmon begin their lives in fresh water, then migrate to the ocean, where they sometimes travel great distances. After a year or more in the ocean, those who survive to maturity return to their stream of origin to spawn and die. Most fishing is directed at the maturing salmon as they return to spawn. Alaskan sockeye spawn far upstream and spend a year or more in lakes and streams before smoltification and migration. The largest numbers of sockeye enter at Bristol Bay or south of the Alaska peninsula and live 2–3 years at sea. Typically, their generation length (the time between spawning of successive generations) is 4–6 years. Alaskan pink and chum originate, in largest numbers, in southeastern Alaska and the northern periphery of the Gulf of Alaska. Pink and most chum spawn near the coastline, migrate quickly into coastal waters, and spend most of their lives in the ocean. Pink salmon consistently return to spawn at the end of their second summer at sea, so they always have a generation length of 2 years. Alaskan chum may live 3–6 years, with a most typical generation length of 4 years. The timing of these life stages is summarized in Table 1.

Table 1. Life-cycle summary of Alaskan salmon.

Species	Freshwater residence	Dominant age at migration	Typical ocean residence	Generation length
Pink	Days to weeks	0	1.6 years	2
Chum	Days to weeks	0	3–6 years	3–6
Sockeye	0–2 years	1 or 2	2–3 years	4–6

Note: Data are from Pearcy (1992) and Alaska Department of Fish and Game (1996).

Deepening of the winter Aleutian low, which is intensified during the warm phase of ENSO or the positive phase of the PDO, leads to weather changes that can affect Alaskan salmon. Warm, moist air is brought into Alaska from the south, increasing coastal rainfall and streamflow and warming the coastal ocean (Mantua et al. 1997). Offshore, anomalous northerly winds increase mixing and horizontal advection, thereby cooling the central and western North Pacific (Miller et al. 1994). In the coastal and marine environments, large-scale climatic forces simultaneously affect salmon stocks from many parts of Alaska.

Materials and methods

Data

Sockeye, pink, and chum salmon have been heavily exploited in Alaska since the 1920s. For large aggregates of stocks, commercial catch data are commonly used as indices of the variability in abundance (Quinn and Marshall 1989; Beamish and Bouillon 1993; Hare and Francis 1995). Statewide catch totals are used in this study to reflect fluctuations in salmon production over a large region. Annual commercial catch figures for the three salmon species were obtained from the Alaska Department of Fish and Game (Rigby et al. 1991, plus annual supplements) and are shown in Fig. 1.

Large-scale hatchery production began in Alaska around 1976 and increased rapidly thereafter (Royce 1989). Climate is generally assumed to affect wild and hatchery fish in the same way. However, abrupt changes in hatchery input could distort our time series models by obscuring climatic impacts or generational patterns in the run sizes of wild salmon. Broken lines in Fig. 1 show the estimated contribution of Alaskan hatcheries to commercial catch, based on estimates by hatchery managers (data provided by M. McNair, Alaska Department of Fish and Game, personal communication). The hatchery production of sockeye is small, increasing catch by only about 5% during 1990–1994. The hatchery contribution to chum catch remained relatively small (around 10%) through 1992 but abruptly jumped to over one third of catch in 1993–1994. Because of this abrupt change, the latter 2 years are not used in developing the chum models in our study. Hatchery production of pinks underwent several successive jumps in 1985, 1987, 1989, and 1990. The estimated hatchery contribution to pink catch averages 21% during 1987–1994. Thus we can expect our pink models to be especially influenced by "noise" in the catch series attributable to changing hatchery production.

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Fig. 1. Alaskan commercial catch, by species. Lower (broken) line shows estimated hatchery production.

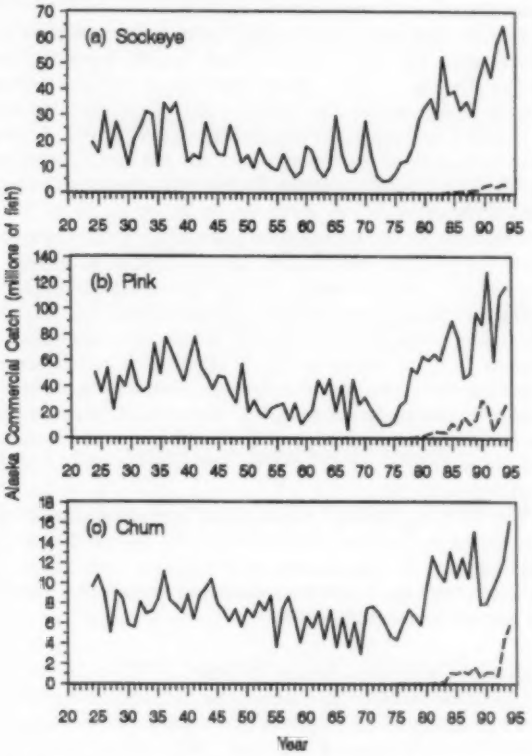
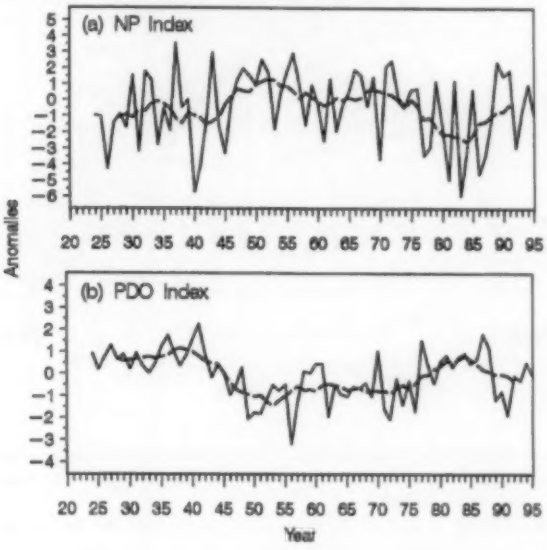


Fig. 2. (*a*) North Pacific index (NPI, in mbars; 1 mbar = 100 Pa) and (*b*) Pacific interdecadal oscillation (PDO) index, for 1924–1995. Both are shown as departures from the 1900–1994 mean. Broken lines are 9-year running means.



To measure variations in the intensity of the Aleutian low pressure system, we use the North Pacific index (NPI) developed by Trenberth and Hurrell (1995). Mean SLP for the "winter months," November through March, is averaged over the region of the North Pacific, which historically has been most affected by winter Aleutian low activity, from 30 to 65°N and from 160°E to 140°W. The NPI was used by Francis and Hare (1994) to identify the timing of climate regime shifts.

The PDO index provides a measure of basin-scale variations in SST. We computed the November–March "winter" PDO index of Mantua et al. (1997), using monthly PDO index data (provided by N. Mantua, University of Washington, personal communication).

The NPI and PDO indices are shown in Fig. 2. (The calendar year label refers to January; thus, the value shown for 1990 is for November 1989 – March 1990.) The broken lines in Fig. 2 are 9-year running means to suggest decadal-scale changes. On the decadal scale, the two indices show somewhat similar fluctuations (of opposite sign), with large shifts in the 1940s and 1970s. The correlation between the two indices is $r = -0.65$.

The surface temperature data that we use to compute regional temperatures were originally assembled for the Intergovernmental Panel on Climate Change to measure long-term changes in global temperatures (IPCC 1992; Trenberth et al. 1992). The dataset combines air temperatures over land and SSTs over oceans into monthly means on a 5 × 5° global grid. The SST data have been adjusted to minimize the effects of potential biases caused by changing measurement methods and inadequate sampling (Bottomley et al. 1990). The temperature measurements are expressed as anomalies from the 1950–1979 mean (in°C). For this study we use the gridded temperature anomalies in the North Pacific area 30–65°N, 115–180°W, averaging the monthly means to obtain seasonal anomalies (seasons: December, January, February; March, April, May; June, July, August; September, October, November). SLP and SST estimates are considered less reliable before 1925 because of sparse data; therefore, our analysis has been limited to 1925–1994.

Methods

Sea surface temperatures display high spatial correlation over large areas of the North Pacific. Cluster analysis (oblique component analysis; SAS Institute Inc. 1989) was applied to the gridded temperature anomalies to identify reasonably homogeneous regions. The mean temperature anomalies in these regions can be expected to reflect large-scale climatic changes, and are used as predictors in the salmon models.

Salmon catch data exhibit correlation over successive years. To control for this autocorrelation, time-series models are developed using ARIMA (autoregressive integrated moving average) modeling techniques (Box and Jenkins 1976). The methods employed are similar to those used by Quinn and Marshall (1989), Hare and Francis (1995), and Hare (1996) in earlier studies of Alaskan salmon. Because the methods are explained in some detail by those authors, they are described only briefly in the Appendix.

The following procedure is used.

(1) Univariate ARIMA models are developed for each time series (1925–1994 for sockeye and pink; 1925–1992 for chum), then step changes near the expected times of regime shifts (late-1940s and late-1970s) are tested. The univariate and step-change models are later used as baselines to determine whether multivariate models provide a better fit.

(2) A modified baseline model for chum is developed using ARIMA transfer function models with pink catch as a predictor (motivated by unexpected autocorrelation patterns found in step 1).

(3) Multivariate models using climate variables as predictors are tentatively identified for each salmon species, using cross-correlation functions.

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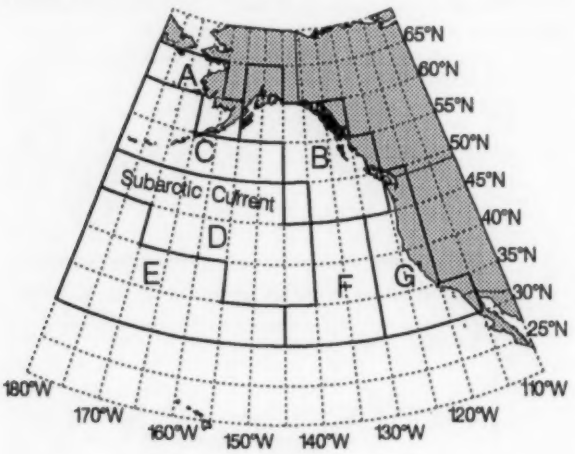
Fig. 3. Temperature regions, determined by cluster analysis on gridded seasonal temperature anomalies.



(4) Transfer-function models are fit to each salmon catch series using predictors identified in step 3, and the best-fitting models are selected.

(5) Finally, to test the robustness of the models, the best model for each species is fit to data for two shorter time periods.

In step 3, multivariate models are tentatively identified by looking at cross-correlation functions (CCFs), which show lagged correlations between pairs of variables. If there is autocorrelation within the individual time series, the data must be "prewhitened." Simple prewhitening (Box and Jenkins 1976) is used when an influence occurs in only one direction, such as that of temperature on salmon. Double prewhitening (Katz 1988) is used when the influence could go in either direction, such as between pink and chum salmon. For models containing step changes, prewhitening to remove the step changes is also used to make interannual relationships more clearly visible in the CCFs (Hare 1996).

In step 4, the model parameters are estimated using maximum-likelihood methods. A significance level $\alpha = 0.05$ is adopted to determine the statistical significance of the predictors. Residuals from each model are checked to assure that no significant autocorrelation remains. Models are compared using Akaike's information criterion (AIC) and the Bayesian information criterion (BIC) to determine which model offers the best fit for each species (equations given in SAS Institute Inc. 1993). In both criteria, the lower value signifies the better model. Sometimes the two criteria lead to different conclusions, so judgment is required.

Stable residual variance (homoscedasticity) is required in regression-based models. To help stabilize the variances, in this study all catch figures (in thousands of fish) are transformed using the equation

$$(1) \qquad Z_t = \ln(\text{catch}_t + 1)$$

where t represents the year of the catch and ln is the natural logarithm. (The 1 is included to allow the possibility of $\text{catch}_t = 0$.) Linear models are fit to the transformed catch data, Z_t, then predictions and forecasts can be transformed back to the original units using the equation:

$$(2) \qquad \text{catch}_t = \exp(Z_t) - 1$$

All computations and model fitting are done using the SAS (Statistical Analysis System) computer package (SAS Institute Inc. 1989, 1993).

Results

Identification of temperature regions

Cluster analysis of the gridded seasonal temperature anomaly data was performed for each season separately and for all seasons together. Cluster patterns in the four seasons do not differ greatly. The analysis identified seven distinct, reasonably homogeneous regions as shown in Fig. 3. Note that many coastal grid squares combine sea and air temperatures, yet are highly correlated with grid squares containing only SSTs.

Regional mean temperature anomalies were computed from the gridded temperature data (weighted according to the area of each grid square). Where data are missing, the regional mean is based on the weighted mean of the nonmissing grid squares. The seasonal temperature anomalies in regions A–E are shown in Fig. 4. (Regions F and G are not expected to affect Alaskan salmon, therefore are not included in this analysis.) The 1977 regime shift is clearly apparent in regions A, B, C, and E. A shift in the 1940s can be seen in regions A, B, D, and E, although the timing of the shift seems to vary.

The NPI and PDO are significantly correlated with winter and spring temperature anomalies in the central North Pacific (regions D and E) and, with opposite sign, along the coastline (regions A and B). In between, region C shows little relation to the NPI or PDO.

Univariate and step-change models

Univariate models for a time series are tentatively identified by looking at its autocorrelation function (ACF), which shows the correlation of the series with itself at successive time lags. The ACF for each salmon species is shown in Fig. 5. All three species show strong patterns of autocorrelation, declining gradually when lagged over 12 years. In addition, there are spikes or humps at regular intervals in the ACF of each species. The slow decline in the ACFs after the first year suggests long-term persistence, which could indicate a step change or a long cyclic pattern. Hare and Francis (1995) found that the best univariate models for their sockeye and pink series were intervention models containing step changes in the late 1940s and late 1970s. Therefore, both autoregressive and intervention models are tested here.

The regular spikes in the ACFs appear to be related to the "generation length" of each species (the time between spawning of successive generations). For sockeye, the ACF rises at lags of 4–5 years and 9–10 years (Fig. 5*a*). The generation length of Alaskan sockeye is typically 4–6 years (Table 1); therefore, a cyclic dominance in run sizes in particular years tends to create autocorrelation at intervals of about 5 years. The damped sine wave appearance of the ACF suggests the use of a "seasonal" autoregressive model (Box and Jenkins 1976) to represent the ripple effect of successive generations in populations that have a fairly consistent generation length. We refer to the seasonal AR models as "generational" models.

Pink salmon consistently have a generation length of 2 years, which shows clearly in their ACF (Fig. 5*b*). Alaskan chum live 3–6 years, with a most typical generation length of 4 years (Quinn and Marshall 1989; Alaska Department of Fish and Game 1996). Surprisingly, in the ACF for chum

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Fig. 4. Seasonal temperature anomalies in five regions of the Northeast Pacific, shown as departures from the 1950–1979 mean (in °C).

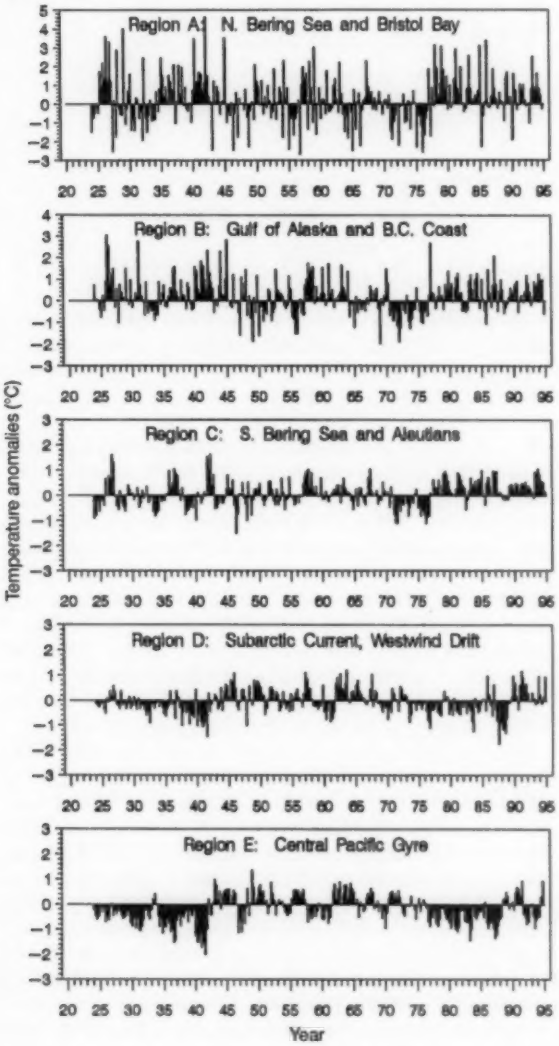
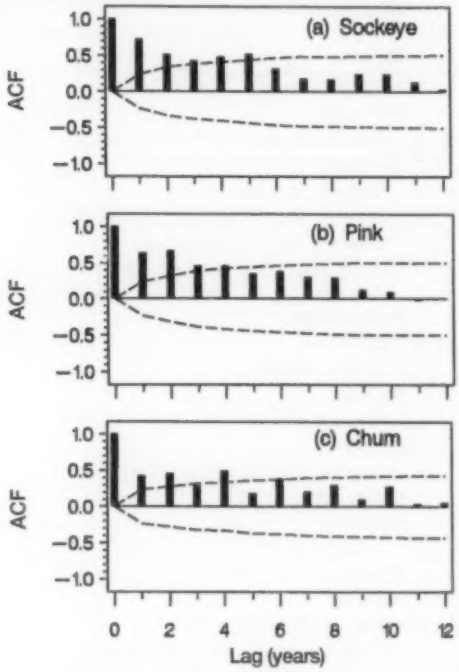

Region A: N. Bering Sea and Bristol Bay
Region B: Gulf of Alaska and B.C. Coast
Region C: S. Bering Sea and Aleutians
Region D: Subarctic Current, Westwind Drift
Region E: Central Pacific Gyre
Temperature anomalies (°C)
Year

Fig. 5. Autocorrelation functions (ACF) for time series of each species, 1925–1994 (N = 70). Broken lines are 2 SEs.



(Fig. 5*c*), although the largest spike is at a lag of 4 years, spikes at 2-year intervals are also apparent. This suggests that the abundance of chum could be related in some way to the abundance of pink salmon. Therefore, in the next section, we examine the cross correlations between pink and chum.

A variety of ARIMA and generational ARIMA model forms were tested for each species. The best in every case, based on comparisons of AIC and BIC, are generational AR models, which include both significant lag-1 autoregression and a significant generational factor (AR_5 for sockeye, AR_2 for pink, and AR_4 for chum). The AIC and BIC values for these univariate models are shown in Table 2, to be used as a basis for comparison with the step-change and multivariate models.

To focus on interannual relationships between catch and climate, we must first control for interdecadal shifts. Step changes in the years 1943–1956 and 1976–1982 were tested in models of each salmon species. In each case the intervention variable was of the form

$$\text{STEP}_y = 0 \text{ for catch years 1925 through } y - 1$$
$$= 1 \text{ for catch years } y \text{ through 1994}$$

where y = 1943, 1944, ..., 1956; 1976, 1977, ..., 1982. The time periods were selected to detect possible responses to the climate regime shift of 1977 (which has been identified fairly precisely in the climate literature) and a less clearly defined regime change in the mid-1940s or early 1950s. All species showed a clear response to the 1977 regime shift. The most significant step changes occured in 1979 for sockeye, 1978 for pink, and 1980 for chum. Changes in catch in these years would be consistent with a change in the marine environment that began affecting smolts in 1977, assuming that the majority of sockeye spent 2 years in the ocean, pink spent 1 year, and chum spent 3 years. Expected ocean residence times in Table 1 indicate these assumptions to be reasonable.

The timing of the earlier regime change is less certain. Francis and Hare (1994) identified it as 1947 based on a significant step change in the NPI in that year and used that as a guide for introducing interventions into their models of sockeye and pink (Hare and Francis 1995). However, step changes in catch are significant in several successive years. Among models containing step changes and ramp-shaped changes, the best-fitting models involved step changes in 1951 for sockeye and 1950 for pink. From this, one might

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Table 2. Comparison of models assuming environmental change in (A) 1947 and 1977 or (B) 1949 and 1977.

(A) Environmental changes in 1947 and 1977.				
Model	No. of residuals	AIC	BIC	R^2
Sockeye salmon				
Univariate				
AR(1), $AR_5(1)$	70	71.6	78.3	0.641
Baseline				
Step 49, step 79	70	59.5	70.7	0.701
Step 49, step 79, sum TC_{t-2}, sum TC_{t-3}	67	50.9	66.4	0.742
Step 49, step 79, sum TA_{t-5}	65	47.8	60.8	0.752
Best-fit				
Step 49, step 79, sum TA_{t-5}, sum TC_{t-2}, sum TC_{t-3}	65	39.5	54.7	0.783 (+0.082)
Pink salmon				
Univariate				
AR(1), $AR_2(1)$	70	85.8	92.6	0.536
Baseline				
Step 48, step 78	70	74.7	83.7	0.604
Best-fit				
Step 48, step 78, spring TD_{t-2}	68	70.4	81.5	0.632 (+0.028)
Chum salmon (1925–1992)				
Univariate				
AR(1), $AR_4(1)$	68	9.7	16.4	0.402
$PINK0_{t-2}$	66	−12.4	−3.6	0.575
Baseline				
Step 80, $PINK0_{t-2}$	66	−13.2	−4.5	0.576
$PINK0_{t-2}$, spring TB_{t-3} (step 80 ns)	65	−23.1	−12.2	0.645
Region B′ (four coastal grid squares) best fit				
Step 80, $PINK0_{t-2}$, spring TB'_{t-3}	65	−29.8	−18.9	0.678 (+0.102)
(B) Environmental changes in 1949 and 1977.				
Sockeye salmon				
Step 51, step 79	70	58.6	69.9	0.705
Step 51, step 79, sum TA_{t-5}, sum TC_{t-2}, sum TC_{t-3}	65	36.4	53.8	0.797
Pink salmon				
Step 50, step 78	70	70.2	79.2	0.629
Step 50, step 78, spring TD_{t-2}	68	67.0	78.1	0.650

Note: Better fit is obtained for sockeye and pink if an environmental change is assumed in 1949 rather than 1947. ns, not significant.

infer that an environmental change affected the largest numbers of smolts of both species beginning in 1949, rather than 1947. (Changes in 1949 for sockeye and 1948 for pink are also significant, only less so; hence, this does not contradict the Francis and Hare results. Rather, it suggests that the regime change of the 1940s may have affected ocean conditions more gradually or unevenly than the abrupt shift in 1976–1977. A further indication that the 1940s shift was more gradual is found in univariate models of the PDO index. The most significant PDO step changes are in 1943 and 1977; however, an even better fit to the PDO index is obtained using a ramp-shaped linear change between 1941 and 1949, together with the 1977 step change.) For consistency with the work of Hare and Francis, we have chosen to use the regime-shift dates that they identified, 1947 and 1977, although all of our models perform somewhat better when based on a 1949 environmental shift (as shown in Table 2B).

The introduction of step changes offers an improvement over the univariate models for all three species, based on reductions in the AIC and BIC (Table 2A). For sockeye and pink, the step-change models will serve as baselines for evaluating the climatic predictors.

Baseline model for chum

The unexpectedly strong autocorrelation in the chum series at 2-year intervals (Fig. 5*c*) suggests a possible relationship with pink salmon. The cross-correlation function (CCF) between chum and pink, after double prewhitening, is shown in Fig. 6. Significant correlation ($r = 0.52$) is apparent at a lag of 2 years; that is, the chum catch in year t is positively correlated with the pink catch in year $t - 2$. (There appears to be no corresponding effect of chum on pink, i.e., the negative lags are not statistically significant within the pinks' 2-year generation length.)

The pink series, centered on its 1925–1977 mean and designated PINK0, was used as a predictor in transfer-function models for chum. The 2-year lag ($PINK0_{t-2}$) gave the best fit; including a step change in 1980 produced a small additional improvement (Table 2A). Step changes during 1943–1956 were tested, but none were statistically significant. It

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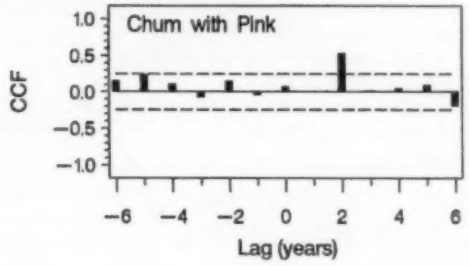
Fig. 6. Cross-correlation function (CCF) of chum and pink series, 1925–1992, after double prewhitening. Broken lines are 2 SEs. (Pink catch leads in positive lags.)



appears that pink catch can be a highly significant predictor of chum catch; therefore, for chum the "pink-predictor" model is used as the baseline for evaluating the climatic predictors.

Identification of multivariate models

The NPI, the PDO index, and temperatures in regions A–E (TA–TE, respectively) are considered as possible predictors of yearly catch. Regions A, B, and C encompass the area most inhabited by Alaskan salmon in the marine phase of life. Regions D and E are included because they are located near the center of influence of the PDO and are likely to reflect large-scale ocean conditions influenced by the Subarctic Current. Winter, spring, and summer temperatures are treated separately for each region; thus, 15 temperature series (5 regions × 3 seasons) are potential predictors.

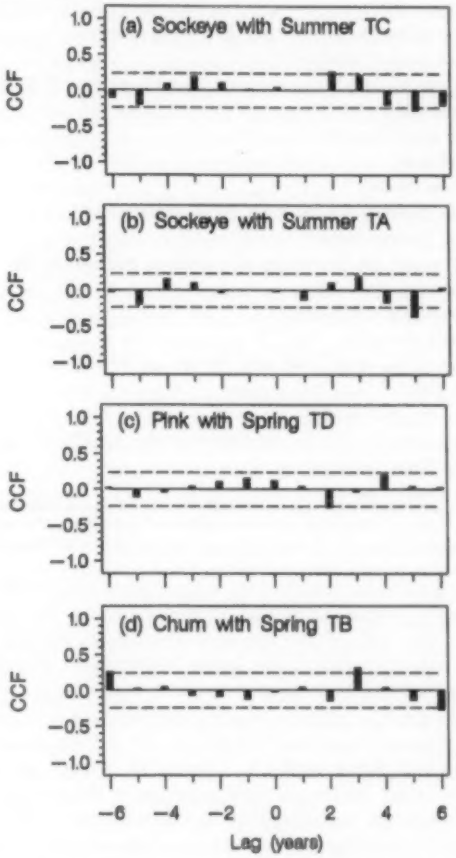
To identify tentative models for each salmon species, CCFs were computed between the catch series and each of the 17 predictors. Simple prewhitening and prewhitening for step changes were applied as needed. (Note that prewhitened series are used only to compute CCFs for model identification; in fitting the final models, the original data are used.) Significant cross correlations beyond the maximum generation length of a given species were assumed to be spurious. It was found that none of the three salmon species are significantly cross-correlated with the climate indices, NPI and PDO. However, catch is significantly cross-correlated with temperature anomalies in several regions: sockeye with regions A, B, and C in spring and summer; pink with region D in spring; and chum with regions A and B in spring. The CCFs of the predictors that ultimately gave the best fit for each species are shown in Fig. 7.

Fitting of multivariate models

For each salmon series, the regional temperature anomalies identified above were added to the baseline models (one temperature series at a time, lagged as indicated by the significant cross correlations). Improvements in fit of the models are apparent from the reductions in the AIC and BIC in Table 2. Equations of the baseline and best-fit models are shown in Table 3. Each species will be discussed in turn.

Sockeye catch is related to temperature anomalies in region C, both 2 and 3 years before the fish are caught. Spring and summer temperatures at lag 2 give about equally good model results, but only summer temperatures are significant at lag 3. (Spring and summer TC are correlated, $r = 0.69$; therefore, one may serve as a surrogate for the other to some extent.) The timing of the relationship corresponds to the first spring and summer after ocean entry, since most sockeye spend 2 or 3 years in the ocean before returning to spawn. The signs of the fitted coefficients are positive (Table 3), indicating that warmer temperatures correspond to higher catch.

Fig. 7. Cross-correlation functions (CCF) of each species with the most significant climatic predictors, 1925–1994, after prewhitening for step changes. Broken lines are 2 SEs. (Temperatures lead in positive lags.)



Sockeye catch is also related to summer temperature anomalies in regions A and B, 5 years before the fish are caught, with region A giving the better model fit. (Summer TA and TB are correlated, $r = 0.63$.) Since the typical generation length of Alaskan sockeye is 5 years, this suggests an association with the spawning time of the parent generation. The sign of the fitted coefficient is negative (Table 3) indicating that, after controlling for interdecadal shifts in mean catch, cooler summer temperatures tend to lead to enhanced sockeye catch 5 years later. The best-fitting sockeye model (Table 2) includes both summer TA (at lag 5) and summer TC (at lags 2 and 3).

Pink salmon models show a statistically significant relationship between pink catch in year t and spring temperature anomalies in region D in year $t - 2$. Given that pinks have a

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Table 3. Best univariate and multivariate time series models.

Species and model	Equation
Sockeye salmon	
Univariate model	$SOCKEYE_t = 9.93 + (1 - 0.69B)^{-1}(1 - 0.51B^5)^{-1}a_t$
Baseline model (with 1949 and 1979 step changes)	$SOCKEYE_t = 9.88 - 0.49STEP_{49} + 1.21STEP_{79} + n_t$
Baseline residual model	$n_t = (1 - 0.35B)^{-1}(1 - 0.42B^5)^{-1}a_t$
Best-fit model (predictors are step changes, summer TA, and summer TC)	$SOCKEYE_t = 9.99 - 0.68STEP_{49} + 1.24STEP_{79} - 0.26TASUM_{t-5} + 0.24TCSUM_{t-2} + 0.22TCSUM_{t-3} + n_t$
Best-fit residual model	$n_t = (1 - 0.26B^5)^{-1}a_t$
Pink salmon	
Univariate model	$PINK_t = 10.61 + (1 - 0.43B)^{-1}(1 - 0.55B^2)^{-1}a_t$
Baseline model (with 1948 and 1978 step changes)	$PINK_t = 10.75 - 0.72STEP_{48} + 1.15STEP_{78} + n_t$
Baseline residual model	$n_t = (1 - 0.20B^2)^{-1}a_t$
Best-fit model (predictors are step changes and spring TD)	$PINK_t = 10.68 - 0.60STEP_{48} + 1.02STEP_{78} - 0.29TDSPR_{t-2} + n_t$
Best-fit residual model	$n_t = (1 - 0.33B^2)^{-1}a_t$
Chum salmon (1925–1992)	
Univariate model	$CHUM_t = 8.92 + (1 - 0.41B)^{-1}(1 - 0.55B^4)^{-1}a_t$
Baseline model (with 1980 step change and PINK0 as predictors)	$CHUM_t = 8.80 + 0.26STEP_{80} + 0.25PINK0_{t-2} + n_t$
Baseline residual model	$n_t = (1 - 0.30B^4)^{-1}a_t$
Best-fit model (predictors are 1980 step change, PINK0, and spring TB′ (coastal))	$CHUM_t = 8.79 + 0.23STEP_{80} + 0.20PINK0_{t-2} + 0.097TB'SPR_{t-3} + n_t$
Best-fit residual model	$n_t = (1 - 0.41B^4)^{-1}a_t$

Note: Data and predictors are as described in Table 2. Coefficients on the predictors are significant at the $\alpha = 0.05$ level. B, Backshift operator; a_t, independent random shocks (white noise), as described in the Appendix.

2-year generation length, this suggests an association with the parent generation similar to that found in the sockeye model. The sign of the fitted coefficient is negative (Table 3), indicating that cooler spring temperatures in region D tend to lead to enhanced pink catch 2 years later.

Chum catch is related to spring temperatures in regions A and B, 3 years before the fish are caught, with region B giving a slightly better model fit. (Spring TA and TB are correlated, $r = 0.62$. Although chum originate in the coastal areas of both regions, catch usually is substantially larger in region B.) The timing would correspond to the time of ocean entry for those chum whose generation length is 4 years (the most typical).

Because of the scale of our temperature grid, region B contains a substantial amount of land area and the temperatures TB are based on both air temperature and SST. Questions about the effect of mixing the two led us to separate region B into coastal and ocean segments and try temperatures from the two segments in the chum model. Temperatures in the coastal segment gave a better fit, and those in the ocean segment a poorer fit, than those from the region as a whole. The coastal segment consists of four grid squares on the southern coast of Alaska (55–60°N, 130–140°W and 60–65°N, 145–155°W, including southeastern Alaska, Prince William Sound, and Cook Inlet) and has been designated TB′ in Tables 2 and 3. Thus, temperature anomalies along the southern Alaska coastline, lagged by 3 years, are the most effective in explaining the interannual variability in chum catch. The relationship is positive (Table 3), indicating that warmer temperatures tend to correspond to higher catch.

The coefficient of determination, R^2, tells us the proportion of variance in the catch that is explained by the model. This provides a useful interpretation of how much a predictor contributes to the model. The change in R^2 when temperature is included as a predictor is shown in parentheses in Table 2. In comparison with the baseline models, inclusion of the best-fitting temperature anomaly series increases R^2 by 0.08 for sockeye, 0.03 for pink, and 0.10 for chum. Step changes to control for interdecadal regime shifts are included in all the baseline and multivariate models; therefore, the increases in R^2 represent interannual variability that is explained by the regional temperatures.

Models for different time periods (1925–1977 and 1949–1994)

Are the above models robust; that is, are the same predictor variables significant in different time periods, and do the best-fitting models maintain a similar form? It would be desirable to fit separate models for each of the three climate regime periods to determine unique characteristics and, perhaps, detect differences in fishery dynamics between high- and low-production regimes. Unfortunately, the intervals between the regime changes are too short for separate time-series analyses. To build an ARIMA model, it is desirable to have at least 50 observations (Pankratz 1983).

The question of robustness can be partially answered, however, by fitting the best models to two overlapping intervals, 1925–1977 and 1949–1994 (each containing one step change), to determine whether the predictors are statistically significant in each. The two time intervals present somewhat different issues. In the earlier period, the time and shape of the regime shift are not clearly defined; also, data collected before 1949 are considered less reliable and coverage is less complete. Toward the end of the later period, hatcheries contribute increasingly to total catch, and Alaskan salmon abundance increases so much that age and size at maturity begin to be affected (Bigler et al. 1996).

The best models from the full time period were fit to data

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in the two shorter intervals. Coefficient estimates change somewhat between the two intervals, but the changes are small. All predictors remain significant at the $\alpha = 0.05$ level. The only major difference between models of the two time intervals is in the residual model for sockeye, where an AR(1) term is required in the later period.

Discussion

These models indicate that salmon abundance is related to interannual variability in ocean temperatures at two distinct times in the life cycle: shortly after ocean entry and shortly before spawning. For sockeye and chum, temperature appears to be an important indicator of survival in the first few months after ocean entry. Warmer (cooler) than normal temperatures are associated with higher (lower) catch a few years later. The locations of the significant temperature anomalies (region C for sockeye, region B for chum) correspond to the expected migration patterns in the first seasons at sea shown by Pearcy (1992, Fig. 6.1). For chum, the particularly strong significance of spring temperature anomalies close to the coastline (region B′) is in keeping with the tendency for juvenile chum to remain in coastal estuaries for several weeks or even months (Pearcy 1992). (Pink salmon, however, did not show a relationship with temperature at the time of ocean entry. We looked for, but failed to find, any relationship with temperatures in region B where large numbers of pink smolts enter the ocean.)

Ocean temperature anomalies at the time of the return migrations appear to be related to abundance of sockeye and pink in the next generation. Alaskan sockeye catch is particularly strongly related to summer temperatures 5 years earlier in region A, which includes Bristol Bay. Maturing sockeye return from the ocean to freshwater systems during summer (Alaska Department of Fish and Game 1996) with returns of sockeye to Bristol Bay consistently peaking in July (Pearcy 1992). Pink catch is related to spring temperature anomalies 2 years earlier in region D, at the time when the parents were probably farthest out to sea. This relationship is unexpected, since pink salmon are not found in abundance in most of region D. However, pinks from southeastern to southwestern Alaska are known to move north and west along the coast, then turn south in winter and enter the eastward-flowing Subarctic Current, which is a part of region D (Pearcy 1992).

A possible key to the importance of temperature at the time of return migration is found in a study of British Columbia salmon. In two pink stocks and two sockeye stocks, Blackbourn (1993) found that the freshwater survival rate of eggs was related to SSTs experienced by the spawners during January to June of the year of their return migration. Pink stocks from widely separated spawning locations had similar location and timing of the most significant SST data, 45–50°N, 140–150°W, which is at the eastern end of our region D. Blackbourn suggests that temperatures experienced by maturing adult salmon may affect the development of eggs or sperm, leading to the observed association with survival rate of the eggs and fry in fresh water. In both his study and ours, the relationship is negative; that is, warmer than normal temperatures tend to be associated with a lower freshwater survival rate of eggs and a lower catch in the offspring generation. These relationships suggest a need for further research, which is beyond the scope of this study.

Our findings for sockeye augment the work of Hare (1996), who attempted to relate sockeye catch to spring SST in an area bounded by 54–58°N and 154–166°W (mostly in our region A). He found no significant relationships. In our sockeye models, it is summer SST that proves to be most important. At the smolt stage, significant summer temperatures in region C, farther from coastal areas, suggest that juvenile sockeye may leave the coastline rather quickly after ocean entry and that temperatures remain important for several months after entry.

A highly significant positive relationship is found between chum catch and that of pink salmon 2 years before. A high (low) pink catch in year $t - 2$ tends to indicate a higher (lower) than normal chum catch in year t. The timing suggests that a common influence occurred at the time of ocean entry: Because of the difference in generation lengths, pinks caught in year $t - 2$ and most chum caught in year t both entered the ocean in year $t - 3$. Pink catch may serve as a surrogate measure for a set of environmental conditions that favor or suppress the survival of smolts of both species. However, the strong 2-year autocorrelation pattern in chum catch suggests a direct relationship with the abundance of pinks. One possible explanation is offered by Parker (1971), who noted the differences in odd- and even-year chum catch and proposed that larger numbers of pink smolts may act as a buffer against predation for chum smolts entering the ocean at the same time. Regardless of the explanation, the relationship suggests that the pink catch of 2 years before might aid in forecasting chum catch in some areas.

Many things change during a 70-year time span (data collection, fishing methods and management, the dynamics of fish populations, to name a few). Therefore, it is reassuring to find that the fit of our best models is quite similar in two shorter (partially overlapping) time periods. The predictors identified for each species are significant for both 1925–1977 and 1949–1994 and are little affected by the many changes, including the introduction of hatcheries in the latter period.

Note that the step changes in our models divide the sockeye and pink data series into three production regimes, each with a different mean level of abundance. Other authors have related those changes in abundance to large-scale climatic changes related to the Aleutian low pressure system (measured by NPI) and basin-scale SST patterns (measured by PDO) which dominate the North Pacific in winter. Our findings indicate that, within those climate–production regimes, regional fluctuations in spring and summer SST affect salmon as smolts and as returning adults.

In the work of Hare and Francis (1995) and Mantua et al. (1997), an open question remained as to why catch continued to be high in the 1990s, after the winter NPI and PDO indices had reversed sign in 1989 and then returned to near-normal levels. Our models may offer an explanation for two of the salmon species: chum and sockeye smolts are shown to benefit from warmer-than-normal spring and summer temperatures in regions B and C, respectively. Warm anomalies continued in regions B and C through 1995 (Fig. 4), despite the weakening of the Aleutian low and the PDO in winter.

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Our examination of the interdecadal regime shifts in both salmon catch and climate data indicates that the changes in the 1940s were less clearly defined in time and less dramatic in scale than those of opposite direction in the late 1970s. Whether the conditions since 1976 result only from recurring regime shifts in the North Pacific climate or reflect a longer term change in the atmosphere and ocean may be debated. Trenberth and Hoar (1996) present statistical evidence that the change toward more frequent ENSO events beginning about 1976 is unlikely to have occurred by chance in the context of the previous climate record. The distinction may be important for fisheries management. Will another regime change soon shift conditions back to 1950–1975 levels? Or do the recent temperature patterns constitute a new "normal" state for marine organisms in the North Pacific?

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References

Adkison, M.D., Peterman, R.M., LaPointe, M.F., Gillis, D.M., and Korman, J. 1996. Alternative models of climatic effects on sockeye salmon, *Oncorhychus nerka*, productivity in Bristol Bay, Alaska, and the Fraser River, British Columbia. Fish. Oceanogr. **5**: 137–152.

Alaska Department of Fish and Game. 1996. Alaska Department of Fish and Game's wildlife notebook series. [Available online from http://www.state.ak.us/local/akpages/FISH.GAME/notebook/notehome.htm]

Beamish, R.J., and Bouillon, D.R. 1993. Pacific salmon production trends in relation to climate. Can. J. Fish. Aquat. Sci. **50**: 1002–1016.

Bigler, B.S., Welch, D.W., and Helle, J.H. 1996. A review of size trends among North Pacific salmon (*Oncorhynchus* spp.). Can. J. Fish. Aquat. Sci. **53**: 455–465.

Blackbourn, D.J. 1993. Sea surface temperature and the subsequent freshwater survival rate of some salmon stocks: a surprising link between the climate of land and sea. *In* Proceedings of the 9th Annual Pacific Climate (PACLIM) Workshop, 21–24 Apr. 1992. *Edited by* K.T. Redmond and V.L. Tharp. Tech. Rep. No. 34. California Department of Water Resources, Interagency Ecological Studies Program, Pacific Grove. pp. 23–32.

Bottomley, M., Folland, C.K., Hsiung, J., Newell, R.E., and Parker, D.E. 1990. Global ocean surface temperature atlas. U.K. Meteorological Office, Bracknell.

Box, G.E.P., and Jenkins, G.M. 1976. Time series analysis: forecasting and control. Holden-Day, Oakland, Calif.

Brodeur, R.D., and Ware, D.M. 1992. Long-term variability in zooplankton biomass in the subarctic Pacific Ocean. Fish. Oceanogr. **1**: 32–38.

Brodeur, R.D., and Ware, D.M. 1995. Interdecadal variability in distribution and catch rates of epipelagic nekton in the Northeast Pacific Ocean. *In* Proceedings, Symposium on Climate Change and Northern Fish Populations, 19–24 Oct. 1992, Victoria, B.C. *Edited by* R.J. Beamish. Can. Spec. Publ. Fish. Aquat. Sci. 121. pp. 329–356.

Ebbesmeyer, C.C., Cayan, D.R., McLain, D.R., Nichols, F.H., Peterson, D.H., and Redmond, K.T. 1990. 1976 step in the Pacific climate: forty environmental changes between 1968–1975 and 1977–1984. *In* Proceedings of the Seventh Annual Pacific Climate (PACLIM) Workshop, April 1990. *Edited by* J.L. Betancourt and V.L. Tharp. Tech. Rep. No. 26. California Department of Water Resources, Interagency Ecological Studies Program, Pacific Grove. pp. 115–126.

Francis, R.C., and Hare, S.R. 1994. Decadal-scale regime shifts in the large marine ecosystems of the Northeast Pacific: a case for historical science. Fish. Oceanogr. **3**: 279–291.

Hare, S.R. 1996. Low frequency climate variability and salmon production. Ph.D. thesis, University of Washington, Seattle.

Hare, S.R., and Francis, R.C. 1995. Climate change and salmon production in the Northeast Pacific Ocean. *In* Proceedings, Symposium on Climate Change and Northern Fish Populations, 19–24 Oct. 1992, Victoria, B.C. *Edited by* R.J. Beamish. Can. Spec. Publ. Fish. Aquat. Sci. No. 121. pp. 357–372.

Intergovernmental Panel on Climate Change (IPCC). 1992. Climate change (1992). *Edited by* J.T. Houghton, B.A. Callander, and S.K. Varney. Cambridge University Press, Cambridge, U.K.

Katz, R.W. 1988. Use of cross correlations in the search for teleconnections. J. Climatol. **8**: 241–253.

Mantua, N.J., Hare, S.R., Zhang, Y., Wallace, J.M., and Francis, R.C. 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. Bull. Am. Meteorol. Soc. **78**: 1069–1079.

Miller, A.J., Cayan, D.R., Barnett, T.P., Graham, N.E., and Oberhuber, J.M. 1994. The 1976–77 climate shift of the Pacific Ocean. Oceanography, **7**: 21–26.

Pankratz, A. 1983. Forecasting with univariate Box–Jenkins models: concepts and cases. Wiley & Sons, New York.

Parker, R.R. 1971. Size selective predation among juvenile salmonid fishes in a British Columbia inlet. J. Fish. Res. Board Can. **28**: 1503–1510.

Pearcy, W.G. 1992. Ocean ecology of North Pacific salmonids. University of Washington Press, Seattle.

Quinn, T.J., and Marshall, R.P. 1989. Time series analysis: quantifying variability and correlation in SE Alaska salmon catches and environmental data. *In* Effects of ocean variability on recruitment and an evaluation of parameters used in stock assessment models. *Edited by* R.J. Beamish and G.A. McFarlane. Can. Spec. Publ. Fish. Aquat. Sci. 108. pp. 67–80.

Rigby, P., McConnaughey, J., and Savikko, H. 1991. Alaska commercial salmon catches, 1878–1991. Reg. Info. Rep. No. 5J91-16, Alaska Department of Fish and Game, Juneau.

Royce, W.F. 1989. Managing Alaska's salmon fisheries for a prosperous future. Fisheries (Bethesda), **14**(2): 8–13.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6. 4th ed. Vol. 2. SAS Institute Inc., Cary, N.C.

SAS Institute Inc. 1993. SAS/ETS user's guide, version 6. 2nd ed. SAS Institute Inc., Cary, N.C.

Trenberth, K.E., and Hoar, T.J. 1996. The 1990–1995 El Niño – Southern Oscillation event: longest on record. Geophys. Res. Lett. **23**:57–60.

Trenberth, K.E., and Hurrell, J.W. 1994. Decadal atmosphere–ocean variations in the Pacific. Clim. Dyn. **9**: 303–319.

Trenberth, K.E., and Hurrell, J.W. 1995. Decadal coupled atmosphere–ocean variations in the North Pacific Ocean. *In* Proceedings, Symposium on Climate Change and Northern Fish Populations, 19–24 Oct. 1992, Victoria, B.C. *Edited by* R.J. Beamish. Can. Spec. Publ. Fish. Aquat. Sci. No. 121. pp. 15–24.

Trenberth, K.E., Christy, J.R., and Hurrell, J.W. 1992. Monitoring global monthly mean surface temperatures. J. Clim. **5**:1406–1423.

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Venrick, E.L., McGowan, J.A., Cayan, D.R., and Hayward, T.L. 1987. Climate and chlorophyll *a*: long-term trends in the central North Pacific Ocean. Science (Washington, D.C.), **238**: 70–72.

Zhang, Y., Wallace, J.M., and Iwasaka, N. 1996. Is climate variability over the North Pacific a linear response to ENSO? J. Clim. **9**: 1468–1478.

Zhang, Y., Wallace, J.M., and Battisti, D.S. 1997. ENSO-like interdecadal variability: 1900–93. J. Clim. **10**: 1004–1020.

Appendix: ARIMA modeling

ARIMA (autoregressive integrated moving average) modeling (Box and Jenkins 1976) provides stochastic methods to analyze time series whose observations are equally spaced in time but not independent.

An autoregressive (AR) model represents the current value of a process z_t as a linear combination of a finite number of previous values of the process and an independent random shock a_t, i.e.:

$$z_t = \phi_1 z_{t-1} + \phi_2 z_{t-2} + \ldots + \phi_p z_{t-p} + a_t$$

The shocks, a_t, are "white noise," randomly drawn from a normal distribution whose mean is zero. In Box and Jenkins' notation, an AR(p) model is an autoregressive model of order p and is written

$$(1 - \phi_1 B - \phi_2 B^2 - \ldots - \phi_p B^p) z_t = a_t$$

where B is the "backward shift" operator defined by $Bz_t = z_{t-1}$, hence $B^k z_t = z_{t-k}$

Many time series exhibit periodic behavior associated with seasonal patterns, for example, the annual business cycle evident in monthly sales data. Salmon catch data often show periodic patterns reflecting a correlation between the size of the parental and offspring generations, with period length corresponding to the generation length of a given species.

Such periodic patterns can be represented parsimoniously using seasonal ARIMA models (Box and Jenkins 1976, Ch. 9), which have a multiplicative form. For example, in monthly data a seasonal autoregressive model containing both second-order autoregression and a periodicity of 12 months might have the form:

$$(1 - \phi_1 B - \phi_2 B^2)(1 - \Phi B^{12}) z_t = a_t.$$

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Timing of habitat shift by Atlantic salmon parr from fluvial to lacustrine habitat: analysis of age distribution, growth, and scale characteristics

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Abstract: This study investigated the timing of a habitat shift from fluvial to lacustrine habitat of Atlantic salmon, *Salmo salar*, parr in a subarctic river in northern Finland and whether the individuals that shifted already had the best growth rate prior to the shift or, alternatively, whether the shift was triggered by competition-induced growth depression. The age distribution of parr indicated that most of the fish that shifted belonged to the age group 2+ and the proportion of 1+ fish was less than 3%. However, the analyses of somatic growth and scale characteristics reflected an earlier shift, at the age of 1+, for the greater part of the lacustrine fish. The growth of lacustrine fish exceeded that of their fluvial counterparts after the first year of life. Based on the growth of the first year, no indications that the lacustrine parr were recruited from the fastest growing part of the population were found. However, the growth patterns during the second year may indicate that fluvial parr with the fastest growth at age 1+ may be those that shift at age 2+. Some evidence of growth depression in fluvial fish was found.

Résumé : Dans le cadre de cette étude, nous avons tenté de déterminer à quel moment les tacons du saumon atlantique effectuent la transition de l'habitat fluvial à l'habitat lacustre dans une rivière subarctique du nord de la Finlande; nous souhaitions également vérifier si les tacons qui effectuaient cette transition présentaient déjà les taux de croissance les plus élevés avant la transition, ou si cette transition était plutôt déclenchée par un ralentissement de la croissance causé par la compétition. L'étude de la répartition des tacons en fonction de l'âge a révélé que la majorité des poissons ayant effectué la transition appartenaient au groupe d'âge 2+ et que la proportion de tacons du groupe 1+ était inférieure à 3 %. Toutefois, des analyses de la croissance somatique et l'examen des caractéristiques des écailles ont indiqué que la transition s'était effectuée plus tôt, à l'âge 1+, chez la majorité des poissons vivant en milieu lacustre. La croissance des poissons passés au milieu lacustre était supérieure à celle des poissons demeurés en milieu fluvial après la première année de vie. Après avoir examiné les taux de croissance durant la première année, nous n'avons trouvé aucune indication nous permettant de croire que les tacons vivant en milieu lacustre provenaient de la portion de la population présentant la croissance la plus rapide. Toutefois, l'évolution de la croissance au cours de la deuxième année pourrait indiquer que les tacons présentant en milieu fluvial la plus forte croissance à l'âge 1+ pourraient être ceux qui effectuent la transition à l'âge 2+. Certaines de nos observations témoignent d'un ralentissement de croissance chez les poissons demeurés en milieu fluvial.

[Traduit par la Rédaction]

Introduction

The spatial distribution of animals in a heterogeneous environment has been widely modelled, starting from the ideal free distribution (IFD) model of Fretwell and Lucas (1970). The basic assumption has usually been that habitat suitability declines with increasing population density. Furthermore, animals distribute themselves so that no individual can benefit from shifting to another habitat, and the proportion of individuals in each habitat should match the proportional profitability of that habitat (Parker 1974). Other models may incorporate unequal competitive ability and inhibition of immigrants through territoriality and dominance (Parker and Sutherland 1986; Grand 1997). A shift of an individual to another habitat may be triggered by increasing densities of conspecific or heterospecific competitors (Rodríguez 1995), or by a competitive bottleneck of a certain size group with depressed growth rates (Näslund et al. 1993). Alternatively, the shifting individuals may be neither the least successful nor the least fit ones (Massot and Clobert 1995) but, rather, those with the fastest individual growth that are facing the limitations of the present habitat (Huntingford et al. 1992; Halvorsen 1996).

Although juvenile anadromous Atlantic salmon, *Salmo salar*, is a typical stream dweller, there is growing evidence that it also uses lacustrine habitat prior to emigrating to the sea. Lacustrine habitat serves as a secondary (in time) nursery area after emergence and early residence in running wa-

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ter. Shifts to a lacustrine habitat are especially well known in both landlocked and anadromous strains of Atlantic salmon in Newfoundland, Canada (Hutchings 1986; Ryan 1986; O'Connell and Ash 1993; Dempson et al. 1996; Erkinaro and Gibson 1997), but they have also been recently documented in Europe for anadromous strains in Iceland (Einarsson et al. 1990), northern Finland (Erkinaro et al. 1995), northern Norway (Halvorsen and Jørgensen 1996), and Ireland (Matthews et al. 1997).

A shift from a fast-flowing, shallow riffle to a lake with standing water is a substantial change for a young salmon, including potential changes in predation threat (Gibson et al. 1993; Erkinaro and Gibson 1997) and lack of need to maintain position against the current. It also includes a change in feeding strategy, from a primary "sit-and-wait" mode of a fluvial fish to a "cruising" mode of a lacustrine dweller (Erkinaro et al. 1998), and may even lead to piscivory (Halvorsen 1996; Erkinaro et al. 1998). Salmon parr may also face different fish species assemblages in lakes relative to those in the fluvial environment (Pihlaja et al. 1998), although an important prerequisite for successful use of lakes by juvenile salmon is a sparse fish fauna with few or no predatory fishes present (Gibson et al. 1993; O'Connell and Ash 1993), which holds true for the geographical areas mentioned above. An often-documented consequence of a shift is the enhanced growth of the lacustrine fish relative to the stream-dwelling individuals (Hutchings 1986; O'Connell and Ash 1993; Halvorsen 1996; Dempson et al. 1996).

Most typically, lacustrine salmon parr have been shown to represent older age groups than their fluvial conspecifics (Dempson et al. 1996; Halvorsen and Jørgensen 1996). However, only studies by Hutchings (1986), Ryan (1986), and Erkinaro and Gibson (1997) have documented actual migration to lacustrine habitat and, thus, the actual age of shifting individuals, either by trapping the immigrant fish or censusing the lacustrine fish population several times during a year. Other studies in Newfoundland or those carried out in northern European river systems (see above) have described the age or size distribution of fish caught in the lake without any direct information about the timing of the habitat shift. Some of the often-used sampling methods, e.g., gill netting, may also include problems of size selectivity (e.g., Hamley 1975).

The aim of this study was to determine the age when salmon parr shift from fluvial to lacustrine habitats in a subarctic river system in northernmost Finland. The age distribution of the lacustrine parr was investigated and the somatic growth and scale characteristics were examined for changes in growth pattern. We also attempted to find evidence for the hypotheses that (*i*) increased competition and the consequent depression of growth would lead to the habitat shift. An alternative hypothesis is that (*ii*) the dispersers already have the best growth rate prior to the shift.

Materials and methods

Study area

The subarctic River Teno system forms the border between northern Norway and Finland (70°N, 27°E) and is the most productive and one of the largest salmon rivers in both countries (catchment area 16 386 km^2). The River Utsjoki is its largest tributary on the Finnish side of the drainage basin (1652 km^2, 66 km in length; Fig. 1) and has a considerable run of Atlantic salmon (annual catch approximately 1.5–3 t in recent years; unpublished data). The river system is characterized by a large number of lakes; about 93% of the wetted area of the River Utsjoki consists of lakes larger than 1 ha. Juvenile salmon is clearly the dominant fish species in the fluvial stretches of the Utsjoki (Länsman et al. 1998) with only few and much less abundant cohabiting species, namely European minnow, *Phoxinus phoxinus*; bullhead, *Cottus gobio*; brown trout, *Salmo trutta*; burbot, *Lota lota*; and sticklebacks, *Gasterosteus aculeatus* and *Pungitius pungitius*. However, the most abundant fish species in the lakes are whitefish, *Coregonus* sp., and grayling, *Thymallus thymallus*, and these cohabit with the above-mentioned ones. Salmon parr have been found furthest upstream in the Lake Cuoggajávri in lacustrine habitats and at the outlet of the Lake Mierašjávri in fluvial habitats (Fig. 1). Water quality in the River Utsjoki is good in that pH has varied mostly between 6.9 and 7.2, alkalinity between 170 and 290 $\mu equiv.\cdot L^{-1}$, conductivity between 3.2 and 4.6 $mS\cdot m^{-1}$, and total P between 2 and 4 $\mu g\cdot L^{-1}$ at a fluvial sampling site in the Utsjoki in 1990–1991 (Traaen and Huru 1992). Secchi depth is about 5 m in the lakes.

Sampling

Fish sampling in 13 lakes in the main stem of the River Utsjoki was carried out with gill nets (mesh sizes 6–20 mm, knot to knot) between July and September in 1996. Usually 10–25 nets per lake were used simultaneously. The nets were set individually and perpendicular to shore from the shoreline down to a maximum depth of 2–10 m. Catch per unit effort was calculated as the number of fish per 100 m^2 net per night (12 h). The two lowermost lakes of the system, lakes Nisojávri and Máttajávri, (Fig. 1) were sampled monthly (July, August, September; Table 1), but the other lakes were sampled only once. Simultaneous fish samples from the fluvial habitat were collected by electrofishing. Comparisons of salmon parr between fluvial and lacustrine habitats were concentrated into two zones: the lower zone (lakes Máttajávri and Nisojávri), and the upper zone (lakes Geavojávri, Jumbáljávri, Jorbajávri, and Giđđasaijávri; Fig. 1). As there is only negligible fluvial juvenile rearing habitat above the Lake Máttajávri until the outlets of rivers Čársejohka and Geavojohka, the corresponding fluvial samples for the lower zone were collected from a riffle section that joins the lakes Máttajávri and Nisojávri, and this is one of the most productive fluvial habitats for juvenile salmon in the entire system (Fig. 1). The fluvial reference area for the upper part of the system was selected from three stretches of riffles, located just above the four lakes. Since the fluvial area in the upper zone of the Utsjoki main stem is relatively sparse, two major spawning tributaries, rivers Čársejohka and Geavojohka, that run to Lake Geavojávri (Fig. 1), were also chosen to represent the potential natal habitat for the upper lacustrine parr, i.e., there are two fluvial reference areas for the upper part of the river system. The fluvial parr samples were collected on the sampling sites from the lowest 2 km of the tributaries. Information about the population densities of juvenile salmon in the fluvial habitats (August–September 1996) was gathered during monitoring programmes of the Finnish Game and Fisheries Research Institute (Länsman et al. 1998).

Water temperatures of the lower part of the system were measured using both an automatic Control One temperature data logger, which was located in Lake Máttajávri, close to the outlet at a depth of 3 m, and a Lambrecht thermograph situated in a riffle section in the lowest part of the River Utsjoki, 3 km downstream of the Lake Máttajávri. Water temperature in the lake was more stable compared with the river and was 0.5–4°C higher than that of the river throughout late August – September (Fig. 2).

Analysis

A total of 1151 salmon parr (411 lacustrine, 740 fluvial; Table 1) were aged from their scales using a semiautomatic scale reading

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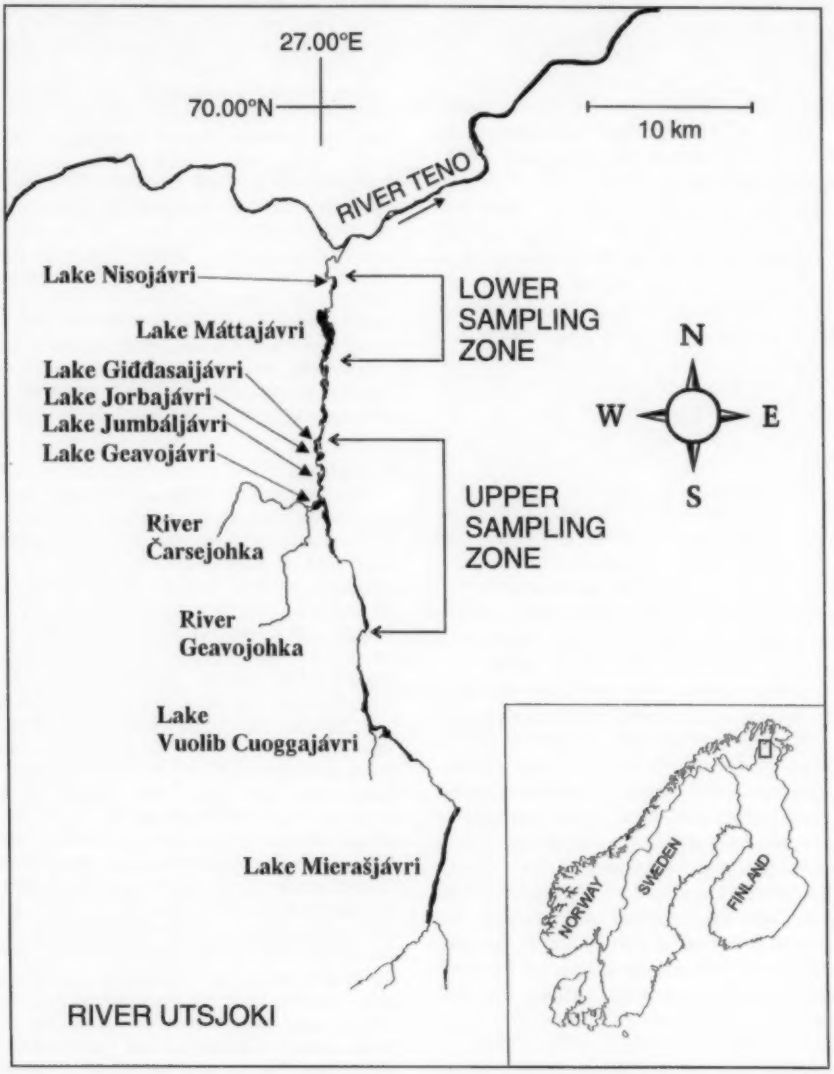
Fig. 1. Study area. Inset shows the location of the two sampling zones in the River Utsjoki.



system with a video camera and image processing system. Interannular distances were measured to the nearest 0.001 mm in addition to counting the number of circuli between the annuli. To track the earlier growth patterns of fish, we used the scale characteristics instead of the traditional back calculation of fish length, since the direct measurements do not include estimation or calculation procedures with underlying assumptions (Francis 1990).

Scale characteristics and body (total) length data were log $(n + 1)$ transformed to meet the assumptions of normality (Lilliefors test) and homogeneity of variances (Levene's test). Comparisons were carried out with one-way ANOVA and Bonferroni test in pairwise comparisons (SPSS, Inc. 1996). Data that did not meet the assumptions were analysed using a nonparametric Kruskall–Wallis test.

The age distribution of salmon parr obtained from the catch was corrected for the selectivity of gill nets of different mesh sizes, as their capture efficiency can be three or four times higher for salmonids of the 15-cm length class than for the 8-cm length class (e.g., Jensen 1995). The maximum selectivities for the mesh sizes used were calculated according to the formula as presented by Jensen (1995, eq. 6) and then used for calculating the estimated true proportions of fish in different length classes in gill nets of different mesh sizes. The length distribution was then transformed to age-classes validated by scale reading. It should be noted that the early life history of juvenile salmon takes place in fluvial habitats and the population of age-0+ and age-1+ fish are obviously not fully represented in lakes. This estimation was thus used as a robust index to obtain the potential, more correct proportions of the youngest lacustrine parr.

Results

Age distribution and length of salmon parr

The most abundant age group in the lacustrine parr catches was 3+, the proportion of which varied between 50 and 75% between the lakes. The next most abundant age

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Table 1. Number of salmon parr samples taken for comparisons of scale characteristics and body lengths; total juvenile salmon densities (mean, range, and number of sites in parenthesis: Länsman et al. 1998) of the fluvial habitats; and catch per unit effort (CPUE) for lacustrine habitats (no. of salmon per 100 m² of gill net, range of CPUE in different lakes).

Sampling site	Month	Age group (*n*) 2+	3+	4+	Mean density (fish/100 m²)	CPUE (fish/100 m² of net)
Rivers						
Utsjoki, lower	July	105	37	—	—	—
	August	74	47	—	128 (61–210, *n* = 5)	—
	September	37	10	—	—	—
Utsjoki, upper	August	35	22	6	13 (0–66, *n* = 29)	—
Čársejohka and Geavojohka	August	165	92	110	30 (5–92, *n* = 13)	—
Lakes						
Máttajávri and Nisojávri	July	52	35	—	—	0.8–18.6
	August	22	167	—	—	11.9–25.9
	September	9	31	—	—	6.2–6.5
Geavojávri–Gidđasaijávri	July–September	6	49	40	—	0.5–7.8

Fig. 2. Daily mean water temperatures of the River Utsjoki (broken line) and the Lake Máttajávri (solid line) in 1993.

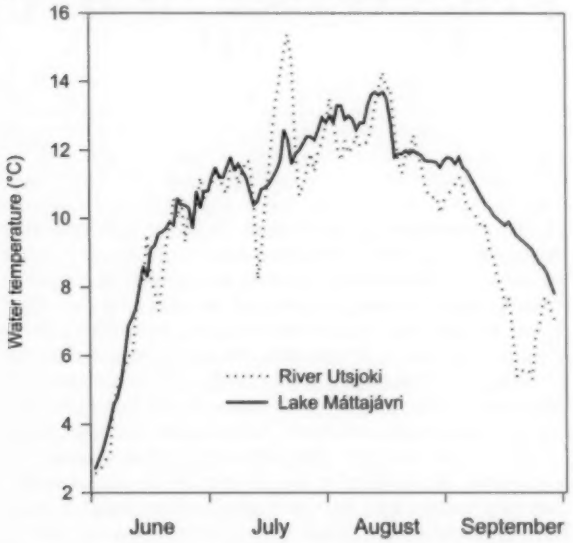
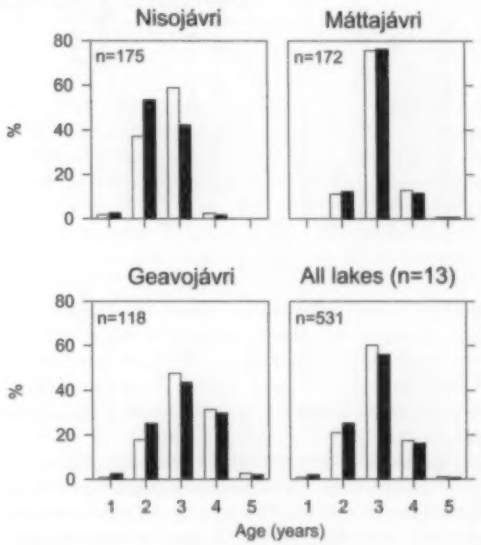


Fig. 3. Age distributions of salmon parr (white bars: actual catch; black bars: corrected for the gill net selectivity, see text) in the lakes of the River Utsjoki.



group was 2+ in the lowermost lake, Lake Nisojávri, but was 4+ in the other lakes. The proportion of 1+ parr was less than 3% in all lakes studied (Fig. 3). When corrected for the gillnet selectivity, the age distribution changed somewhat in favour of the youngest age groups. The proportion of 1+ fish doubled in Nisojávri, and the overall proportions of 1+ and 2+ fish increased (Fig. 3).

Parr from the lakes in the lower part of the Utsjoki (both 2+ and 3+ age groups) were significantly longer than their fluvial counterparts in August and September (2+: $F_{1,94}$ = 63.3 and $F_{1,44}$ = 61.6, $p < 0.001$; 3+: $F_{1,212}$ = 26.2 and $F_{1,39}$ = 39.1, $p < 0.01$, respectively) but not in July (Fig. 4). Similarily, lacustrine parr of the upper reaches of the river were larger than the fluvial parr of the same age in the two tributaries (2+: $F_{2,203}$ = 117.7; 3+: $F_{2,160}$ = 184.9, $p < 0.001$, Bonferroni pairwise tests $p < 0.001$; 4+: Kruskall–Wallis, H = 91.4, $p < 0.001$), but no differences in size were detected between the lacustrine parr and fluvial parr of the upper main stem of the Utsjoki (Fig. 5).

Scale characteristics

The scale characteristics of the lower Utsjoki area revealed similar patterns as seen with the mean lengths of the fish, in that the scale increments (second year: $F_{1,297}$ = 8.8; third year: $F_{1,44}$ = 32.2, $p < 0.001$) and number of circuli (second year: $F_{1,297}$ = 28.2; third year: $F_{1,44}$ = 32.9, $p < 0.001$) of 2+ lacustrine parr exceeded that of the fluvial parr after the first year of life (Fig. 6). In age group 3+, the differences were significant after the second year of life both in scale increments (third year: $F_{1,325}$ = 7.8; fourth year: $F_{1,39}$ = 4.8, $p < 0.05$) and in number of circuli (third year: $F_{1,325}$ = 21.8; fourth year: $F_{1,39}$ = 15.4, $p < 0.001$). The growth after the last full growing season (plus growth) in both age groups

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Fig. 4. Mean (± SD) total lengths of fluvial (squares) and lacustrine (solid circles) salmon parr in the lower part of the River Utsjoki system. Asterisks indicate significant differences between groups within months (ANOVA, $p < 0.01$).

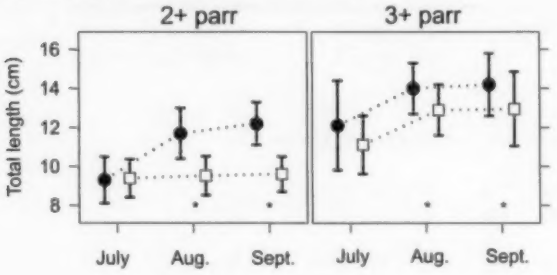
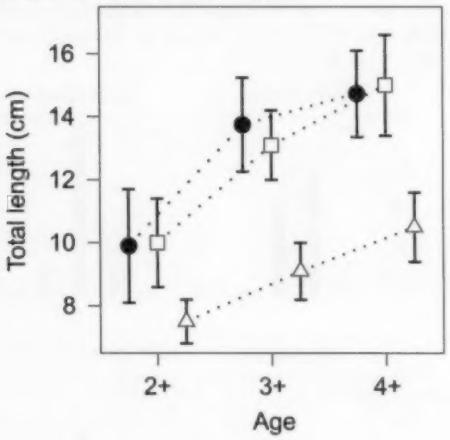


Fig. 5. Mean (± SD) lengths of fluvial (main stem, squares; tributaries, triangles) and lacustrine (solid circles) salmon parr in the upper part of the River Utsjoki system.



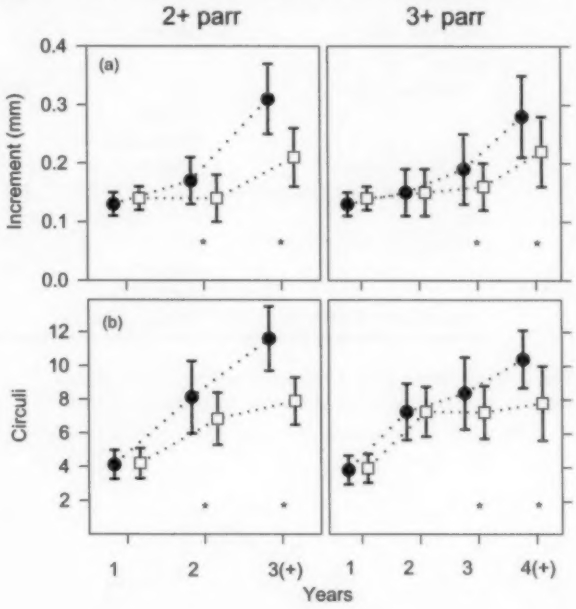
was compared by using the scale samples collected in September.

Differences in scale increments were significant after the first year of life, both between the lacustrine parr of the upper river system and the fluvial parr of the tributaries and between the upper Utsjoki fluvial parr and the tributary parr ($F = 10.6–77.7$, Bonferroni pairwise tests $p < 0.001$; Fig. 7). The number of circuli after the first year of life (years 2, 3, and 4; Fig. 7) were also significantly higher in both the lacustrine parr and the upper Utsjoki fluvial parr compared with those of the tributary parr (five of six cases; $F = 12.4–57.0$, Bonferroni pairwise tests $p < 0.01$). In contrast to the parr from lakes and Utsjoki main stem, the scale increments decreased or stayed at the same level after the first year of life in the tributary parr. There were no differences in the scale characteristics between the upper lacustrine parr and the upper Utsjoki fluvial parr (Fig. 7).

Discussion

The age distribution of salmon parr in the lakes of the River Utsjoki showed the most numerous age-class in the population to be 3+. The youngest age group encountered,

Fig. 6. Yearly growth increments (*a*) and number of circuli (*b*) (mean ± SD) of fluvial (squares) and lacustrine (solid circles) salmon parr in the lower zone of the River Utsjoki system. Asterisks indicate significant differences between groups (ANOVA, $p < 0.001$). +, plus growth of the sampling season.



1+, was represented by fewer than 3% of the fish, even after correcting for gillnet selectivity, and the 2+ group was the second most abundant age-class in the population. This distribution may indicate that most of the parr that shift from fluvial to lacustrine habitat move during their third year of life (age 2+). This is in accordance with the age distributions presented by Dempson et al. (1996) and O'Connell and Dempson (1996) from Newfoundland and by Halvorsen (1996) from northern Norway, which show that age groups 2+ and 3+ are the most abundant in lacustrine salmon parr populations. In addition to the 2+ and 3+ groups, considerable proportions of 4+ parr have also been recorded in some Newfoundland ponds (Ryan 1986), as was also the case in this study. In only few cases have underyearlings moved or been displaced into lakes (Einarsson et al. 1990; O'Connell and Dempson 1996; Erkinaro and Gibson 1997; Matthews et al. 1997).

In addition to the age distribution, the patterns in somatic growth and scale characteristics of salmon parr allow inferences to be made about the timing of their habitat shift from fluvial to lacustrine habitat. The number of 1+ parr caught in the lakes was so low that no direct comparisons of their size between fluvial and lacustrine habitat could be made. However, the scale characteristics of older parr revealed that growth during the first year of life was equal in both habitat types. The earliest divergence in growth patterns took place at the age of 1+, although no differences could be detected until the third year of life (age 2+) in the 3+ group of the lower River Utsjoki. The latter finding could be related to the observations made by Halvorsen (1996) who found that the fastest growing individuals may enter the lakes at the

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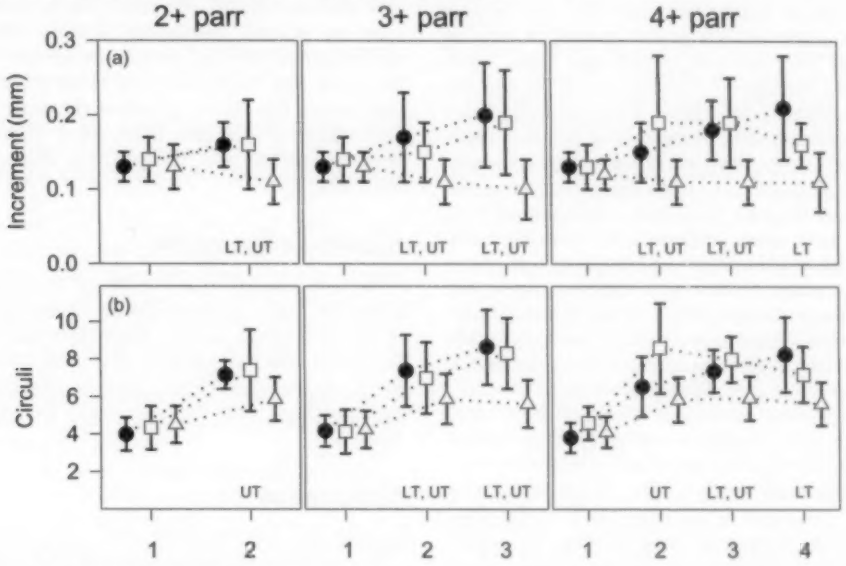
Fig. 7. Yearly growth increments (*a*) and number of circuli (*b*) (mean ± SD) of fluvial (main stem, squares; tributaries, triangles) and lacustrine (solid circles) salmon parr in the upper zone of the River Utsjoki system. Significant differences between the compared pairs are indicated (ANOVA, $p < 0.001$) as, for example, LT (lakes vs. tributaries).



age 1+, but slower growing individuals do so at the age of 2+. It is possible that 3+ fish in the lake include more late immigrants than the 2+ group, i.e., the recently shifted fish may have slower growth during their early life that influence the lower mean values of the entire cohort. However, no evidence is available to support this suggestion. As suggested in earlier studies, the fastest-growing individuals are those that may shift to alternate habitats first (e.g., Huntingford et al. 1992). It is also possible that the need to leave the original habitat may develop for different age groups in different years because of the high yearly and seasonal variation in environmental conditions, e.g., temperature and food availability and their impact on growth rate, typical to northern ecosystems (e.g., Craig and Poulin 1975).

The significantly higher growth of lacustrine parr during their second year of life (age 1+) may reflect a higher proportion of lacustrine immigrants at the age of 1+ than could be found in the gillnet samples. Young parr occupy primarily the shallowest littoral zones of the lakes (O'Connell and Dempson 1996; Halvorsen 1996) and their proportion may be underestimated in the gillnet catch because of their habitat use (Jensen 1995) and the selectivity of the gill nets (Hamley 1975; Jensen 1995). The corrected age distribution showed a higher proportion of 1+ and 2+ fish in the lakes than the actual catch did when estimated by correcting for gillnet selectivity (Jensen 1995). It should be noted that the Fulton's condition factor (Bagenal and Tesch 1978) of lacustrine salmon parr in the Utsjoki was 0.9 ± 0.1 (mean ± SD), $n = 352$ (unpublished data), which is lower than that of brown trout (1.19) from Jensen's (1995) material. Thus, the capture efficiency for the smallest fish may be even poorer than in Jensen's study, and the actual proportion of, for example, 1+ fish could be higher. In some earlier studies, the lakeward-migrating salmon parr consist mainly of 1+ parr (Hutchings 1986; Erkinaro and Gibson 1997), and according to Ryan (1986), the immigrating parr in the summer mainly consist of age groups 1+ and 2+. An alternative explanation for the higher growth of lacustrine parr during their second year of life is that individuals with the best growth rate are most likely to shift to a secondary habitat (Huntingford et al. 1992; Erkinaro and Niemelä 1995; Halvorsen 1996), but they may do it in later years, during the third (2+) or fourth (3+) year of life.

The results of this study confirm the earlier preliminary findings about the enhanced growth of lacustrine salmon parr relative to their fluvial conspecifics in the River Utsjoki system (Erkinaro et al. 1995), supporting results from Newfoundland (Hutchings 1986; O'Connell and Ash 1993; Dempson et al. 1996) and northern Norway (Halvorsen 1996). Furthermore, some stocking experiments of juvenile salmon to lakes have resulted in exceptional growth rates that were greater than those recorded anywhere for salmon parr in fresh water, in that the 1+ parr attaining fork lengths up to 22 cm at the end of their second summer (e.g., Gibson and Haedrich 1988). However, there were no differences in length between lacustrine and fluvial parr in early summer (Fig. 4). Some of the parr in July may have been new recruits to the lake, assuming that most of the shifts take place in spring – early summer (e.g., Hutchings 1986; Erkinaro and Gibson 1997) and cause higher variation within the age group and lack of significant difference in comparisons. The lacustrine parr have stayed one additional growing season in the lakes until September, and thus, the growth difference between the habitats is more pronounced late in the season.

In contrast to the assumptions of the ideal free distribution theory, the suitability of the two habitats seem to be different. Based on the juvenile growth rates, one would predict an increase in fitness if an individual shifts from fluvial to lacustrine habitat. However, the further life-history consequences of the habitat choice in relation to survival and re-

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productive success are unknown (cf. Hutchings 1986; Dempson et al. 1996; Erkinaro et al. 1997).

Where do the lacustrine salmon parr in the River Utsjoki lakes originate? In some studies the origin of lacustrine parr can be determined easily because of the location of lakes at the headwaters of river systems (Hutchings 1986; Ryan 1986; Erkinaro and Gibson 1997). However, in others, the direction of recruitment is not known if the spawning sites are located both upstream and downstream of the lakes (Halvorsen 1996). In this study, there are at least some spawning and fluvial production areas in both the inlet and outlet streams of the lakes. The parr of the two lowermost lakes of the River Utsjoki may come predominantly from the riffle sections between lakes Máttajávri and Nisojávri, and below Nisojávri, as these areas are the most important spawning and fluvial juvenile production areas of the River Utsjoki (Länsman et al. 1998; Pihlaja et al. 1998; Table 1). In addition, the short fluvial stretches upstream from Lake Máttajávri (4.3 ha between Lakes Máttajávri and Geavojávri, mean density 350 juvenile salmon·ha^{-1}, range 0–1400, n of sites = 6; Länsman et al. 1998) are not likely to be able to produce enough recruits for the downstream lakes.

The question about the origin of the lacustrine parr is somewhat more complicated in the upper parts of the river. The lower parts of the tributaries, especially the Geavojohka, have relatively high densities of juvenile salmon (mean 5200·ha^{-1}, range 2800–9200, n of sites = 6), whereas the upper fluvial parts of the Utsjoki main stem have relatively sparse fluvial production area and clearly lower densities (20 ha, mean 1300·ha^{-1}; Table 1 and unpublished data). This may indicate that most of the salmon parr population of Lake Geavojávri and the adjacent lakes below have recruited from the tributaries. On the other hand, the growth patterns of the lacustrine parr closely resemble that of the Utsjoki parr but clearly differ from that of the tributary fish. Since different tributaries of the River Teno system support genetically distinct salmon stocks (Elo et al. 1994), uncovering the origin of the lacustrine parr of the upper Utsjoki lakes might be possible by genetical analysis.

In the case of the upper sampling zone, the results of this study did somewhat support the first hypothesis that the intraspecific competition induced growth depression of a certain size group would drive some individuals to shift their habitat (cf. Näslund et al. 1993). However, other factors might also be involved, such as differences in habitat productivity and food availability (e.g., Gibson and Dickson 1984). The yearly growth increments of the tributary parr were markedly lower than those of the other groups and decreased after the first year of life. However, this was not the case in the Utsjoki main stem parr in either sampling zones. In general, the overall growth of the fluvial fish was comparable or better than in other parts of the River Teno system (Erkinaro and Niemelä 1995).

With regard to the second hypothesis, there were no differences in growth between the future fluvial or lacustrine fish during their first year of life in either the lower or upper Utsjoki, and the same was true for 3+ parr also for the second year of life (when they were of age 1+) in the lower area. However, the growth during the second year was different between the lacustrine and fluvial parr of age 2+ in both upper and lower zones and for 3+ fish in the upper zone. Since the proportion of 1+ parr caught in lakes was low, even after correcting for the selectivity of the gear, the fluvial parr with the fastest growth at age 1+ may be those that shift at age 2+. Therefore, it is concluded that there were no obvious indications that the shifting individuals were the best-growing proportion of the population during their first year of life (0+), but better growth during the second year of life (1+) may reflect enhanced growth of the future lacustrine fish that takes place already in the fluvial environment (see also Erkinaro and Niemelä 1995; Halvorsen 1996).

Acknowledgements

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References

Bagenal, T.B., and Tesch F.W. 1978. Age and growth. *In* Methods for assessment of fish production in fresh waters. 3rd ed. *Edited by* T.B. Bagenal. Blackwell Scientific Publications Ltd, Oxford, U.K. pp. 101–136.

Craig, P.C., and Poulin, V.A. 1975. Movements and growth of Arctic grayling (*Thymallus arcticus*) and juvenile Arctic char (*Salvelinus alpinus*) in a small Arctic stream, Alaska. J. Fish. Res. Board Can. **32**: 48–52.

Dempson, J.B., O'Connell, M.F., and Shears, M. 1996. Relative production of Atlantic salmon from fluvial and lacustrine habitats estimated from analyses of scale characteristics. J. Fish Biol. **48**: 329–341.

Einarsson, S.M., Mills, D.H., and Johannsson, V. 1990. Utilisation of fluvial and lacustrine habitat by anadromous Atlantic salmon, *Salmo salar* L., in an Icelandic watershed. Fish. Res. (Amst.), **10**: 53–71.

Elo, K., Vuorinen, J., and Niemelä, E. 1994. Genetic resources of Atlantic salmon (*Salmo salar* L.) in Teno and Näätämö Rivers, northernmost Europe. Hereditas, **120**: 19–28.

Erkinaro, J., and Gibson, R.J. 1997. Interhabitat migration of juvenile Atlantic salmon in a Newfoundland river system, Canada. J. Fish Biol. **51**: 373–388.

Erkinaro, J., and Niemelä, E. 1995. Growth differences between the Atlantic salmon parr, *Salmo salar*, of nursery brooks and natal rivers in the River Teno watercourse in northern Finland. Environ Biol. Fishes, **42**: 277–287.

Erkinaro, J., Shustov, Y., and Niemelä, E. 1995. Enhanced growth and feeding rate in Atlantic salmon parr occupying a lacustrine habitat in the River Utsjoki, northern Scandinavia. J. Fish Biol. **47**: 1096–1098.

Erkinaro, J., Dempson, J.B., Julkunen, M., and Niemelä, E. 1997. Importance of ontogenetic habitat shifts to juvenile output and life history of Atlantic salmon in a large subarctic river: an approach based on analysis of scale characteristics. J. Fish Biol. **51**: 1174–1185.

Erkinaro, J., Shustov, Y., and Niemelä, E. 1998. Feeding strategies of Atlantic salmon (*Salmo salar* L.) parr occupying lacustrine and fluvial habitats in a subarctic River Teno, northern Finland. Pol. Arch. Hydrobiol. **45**: 259–268.

© 1998 NRC Canada

Francis, R.I.C.C. 1990. Back-calculation of fish length: a critical review. J. Fish Biol. **36**: 883–902.

Fretwell, S.D., and Lucas, H.L., Jr. 1970. On territiorial behaviour and other factors influencing habitat distribution in birds. I. Theoretical development. Acta Biotheor. **19**: 16–36.

Gibson, R.J., and Dickson, T.A. 1984. The effects of competition on the growth of juvenile Atlantic salmon. Nat. Can. **111**: 175–191.

Gibson, R.J., and Haedrich, R.L. 1988. The exceptional growth of juvenile Atlantic salmon (*Salmo salar*) in the city rivers of St. John's, Newfoundland, Canada. Pol. Arch. Hydrobiol. **35**: 385–407.

Gibson, R.J., Stansbury, D.E., Whalen, R.R., and Hillier, K.G. 1993. Relative habitat use, and inter-specific and intra-specific competition of brook trout (*Salvelinus fontinalis*) and juvenile Atlantic salmon (*Salmo salar*) in some Newfoundland rivers. *In* Production of juvenile Atlantic salmon, *Salmo salar*, in natural waters. *Edited by* R.J. Gibson and R.E. Cutting. Can. Spec. Publ. Fish. Aquat. Sci. No. 118. pp. 53–69.

Grand, T.C. 1997. Foraging site selection in juvenile coho salmon (*Oncorhynchus kisutch*): ideal free distributions of unequal competitors. Anim. Behav. **53**: 185–196.

Halvorsen, M. 1996. Lake use by Atlantic salmon (*Salmo salar* L.) parr and other salmonids in northern Norway. Dr. Scient. thesis. University of Tromsø, Tromsø, Norway.

Halvorsen, M., and Jørgensen, L. 1996. Lake-use by juvenile Atlantic salmon (*Salmo salar* L.) and other salmonids in northern Norway. Ecol. Freshwater Fish, **5**: 28–36.

Hamley, J.M. 1975. Review of gillnet selectivity. J. Fish. Res. Board Can. **32**: 1943–1969.

Huntingford, F.A., Thorpe, J.E., Garcia de Leaniz, C., and Hay, W. 1992. Patterns of growth and smolting in autumn migrants from a Scottish population of Atlantic salmon, *Salmo salar* L. J. Fish Biol. **41**(Suppl. B): 43–51.

Hutchings, J.A. 1986. Lakeward migrations by juvenile Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. **43**: 732–741.

Jensen, J.W. 1995. A direct estimate of gillnet selectivity for brown trout. J. Fish Biol. **46**: 857–861.

Länsman, M., Niemelä, E., Kylmäaho, M., Kärki, P., and Moen, K. 1998. Tuloksia Tenojoen ja Näätämöjoen vesistöalueilla vuosina 1996–1997 tehdyistä tutkimuksista [Report of the investigations in the Teno and Näätämö river systems in 1996–1997]. Kala-ja riistaraportteja nro 119. Finnish Game and Fisheries Research Institute, Utsjoki. (In Finnish.)

Massot, M., and Clobert, J. 1995. Influence of maternal food availability on offspring dispersal. Behav. Ecol. Sociobiol. **37**: 413–418.

Matthews, M.A., Poole, W.R., Dillane, M.G., and Whelan, K.F. 1997. Juvenile recruitment and smolt output of brown trout (*Salmo trutta* L.) and Atlantic salmon (*Salmo salar* L.) from a lacustrine system in western Ireland. Fish. Res. (Amst.), **31**: 19–37.

Näslund, I., Milbrink, G., Eriksson, L.-O., and Holmgren, S. 1993. Importance of habitat productivity differences, competition and predation for the migratory behaviour of Arctic charr. Oikos, **66**: 538–546.

O'Connell, M.F., and Ash, E.G.M. 1993. Smolt size in relation to age at first maturity of Atlantic salmon (*Salmo salar*): the role of lacustrine habitat. J. Fish Biol. **42**: 551–569.

O'Connell, M.F., and Dempson, J.B. 1996. Spatial and temporal distributions of salmonids in two ponds in Newfoundland, Canada. J. Fish Biol. **48**: 738–757.

Parker, G.A. 1974. The reproductive behaviour and the nature of sexual selection in *Scatophaga stercoraria* L. (Diptera: Scatophagidae). IX. Spatial distribution of fertilisation rates and evolution of male search strategy within the reproductive area. Evolution, **28**: 93–108.

Parker, G.A., and Sutherland, W.J. 1986. Ideal free distributions when individuals differ in competitive ability: phenotype-limited ideal free models. Anim. Behav. **34**: 1222–1242.

Pihlaja, O., Niemelä, E., and Erkinaro, J. 1998. Introduction and dispersion of the bullhead, *Cottus gobio* L., in a sub-Arctic salmon river in northern Finland. Fish. Manage. Ecol. **5**: 139–146.

Rodríguez, M.A. 1995. Habitat-specific estimates of competition in stream salmonids: a field test of the isodar model of habitat selection. Evol. Ecol. **9**: 169–184.

Ryan, P.M. 1986. Lake use by wild anadromous Atlantic salmon, *Salmo salar*, as an index of subsequent adult abundance. Can. J. Fish. Aquat. Sci. **43**: 2–11.

SPSS, Inc. 1996. SYSTAT version 6.0 for Windows: statistics. SPSS, Inc., Chicago, Ill.

Traaen, T.S., and Huru, H. 1992. Overvåkning av Tanavassdraget 1990–1991. Nor. Inst. Vattenforsk. Rapp. nro. 2757 Norsk Institutt for Vattenforskning, Oslo. (In Norwegian.)

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Morphometric and meristic differences between shallow- and deep-water populations of white hake (*Urophycis tenuis*) in the southern Gulf of St. Lawrence

Thomas Hurlbut and Douglas Clay

Abstract: Linear discriminant function analysis of morphometric and meristic characters was used to assess the extent of differentiation between shallow- (<100 m) and deep- (>200 m) water populations of white hake (*Urophycis tenuis*) from the southern Gulf of St. Lawrence (NAFO Division 4T). Although meristic characters provided some evidence for stock separation, the best statistical separation was obtained with morphometric characters. Morphometric discriminant functions derived from "learning" samples were able to correctly classify 82 and 84% of the "test" samples for both females and males, respectively. A greater relative snout length in fish sampled from along the Laurentian Channel compared with those from the Northumberland Strait area was the primary discriminating character for both sexes. Our morphological evidence and previous tagging and distributional studies suggest that the populations from these two areas represent separate stocks; therefore, the traditional management unit for white hake in NAFO Division 4T may no longer be appropriate.

Résumé : Nous avons effectué une analyse discriminante linéaire d'une série de caractères morphométriques et méristiques afin d'évaluer le degré de différenciation entre la population de merluche blanche (*Urophycis tenuis*) vivant en eau peu profonde (<100 m) et la population vivant en eau plus profonde (>200 m) dans le sud du golfe du Saint-Laurent (division 4T de l'OPANO). Si les caractères méristiques ont révélé d'une façon plus ou moins nette la présence de deux stocks distincts, les caractères morphométriques se sont révélés beaucoup plus efficaces à cet égard. Les fonctions discriminantes morphométriques dérivées des échantillons d'apprentissage ont permis de classifier correctement 82 et 84% des échantillons « expérimentaux » de femelles et de mâles, respectivement. La longueur relative du museau était le principal caractère discriminant, les valeurs étant chez les deux sexes plus élevées chez les spécimens provenant du chenal Laurentien que chez ceux capturés dans le détroit de Northumberland. Nos observations morphométriques et les résultats d'études antérieures de marquage et de répartition donnent à croire que les populations associées à ces deux régions constituent des stocks différents. Il n'est donc plus approprié de maintenir l'unité de gestion qui est actuellement prévue pour la merluche blanche dans la division 4T de l'OPANO.

[Traduit par la Rédaction]

Introduction

The white hake (*Urophycis tenuis*) is a demersal fish that inhabits the continental shelf and upper continental slope of the western Atlantic Ocean. They occur from southern Labrador, and the Grand Bank southward to North Carolina, occasionally straying to deep waters off Florida (Musick 1974). White hake are exploited throughout their geographical range by seasonal fisheries, but the most important directed fishery for this species has occurred in the southern Gulf of St. Lawrence (Northwest Atlantic Fisheries Organization (NAFO) Division 4T).

The current management of marine fish stocks in Atlantic Canada assumes the presence of a single stock per management area (Parsons 1993), with no stock mixing during periods of fishing. Despite the fundamental importance of this concept and the significant local commercial fishery for white hake in the southern Gulf of St. Lawrence, there have been no studies of stock structure in the northwest Atlantic.

A tagging study conducted off eastern Prince Edward Island in the late 1960s suggested that white hake in the southern Gulf of St. Lawrence are distinct from white hake in other areas of the northwest Atlantic (Kohler 1971). Consequently, NAFO Division 4T was adopted as the management unit for white hake in the southern Gulf of St. Lawrence, which are currently assessed and managed as a single stock (Morin and Hurlbut 1994). However, white hake exhibit a disjunct distribution in the southern Gulf during the summer and early fall, occurring either in shallow (<100 m) inshore areas in the vicinity of the Northumberland Strait or in deep (>200 m) water along the Laurentian Channel (Fig. 1*a*)

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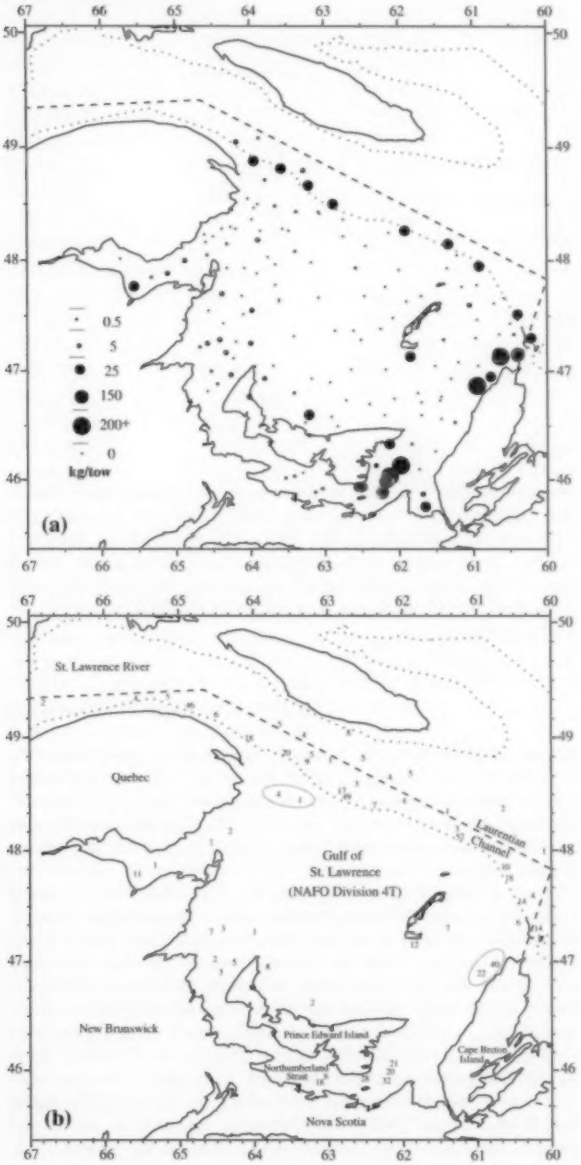
Fig. 1. (*a*) Distribution of white hake catches during the September 1986 demersal fish survey of the southern Gulf of St. Lawrence. (*b*) Sample sites during the two RV *Lady Hammond* surveys of 1986 (numbers indicate the sample size at each location and the ellipses enclose samples from depths between 100 and 200 m). In both panels, the dashed line represents the border of NAFO Division 4T and the dotted line represents the 200-m depth contour.



(Koeller and LeGresley 1981; Clay and Hurlbut 1989; Clay 1991; Morin and Hurlbut 1994). Clay and Hurlbut (1989) and Clay (1991) postulated that these two components may represent completely or partially independent stocks. They referred to the inshore and offshore components as the Strait and Channel components, respectively.

Table 1. Morphometric and meristic characters and their acronyms that were assessed in this investigation.

Character acronym	Description
Morphometric	
PECTBGIR	Pectoral body girth
OTOLHSL	Length of the sagittal otolith (left side)
OTORHSL	Length of the sagittal otolith (right side)
Meristic	
FDORFR	First dorsal fin rays
SDORFR	Second dorsal fin rays
ANALFR	Anal fin rays
PECTFR	Pectoral fin rays (left side)
CAUDFR	Caudal fin rays
TOTVERT	Total vertebrae
ABDVERT	Abdominal vertebrae
EPIRAK	Gill rakers on the epibranchial of the first left arch
CERRAK	Gill rakers on the ceratobranchial of the first left arch

Note: See Fig. 2 for the other morphometric characters.

Our objective was to test the hypothesis that white hake from the Strait and Channel groups represent discrete stocks by assessing morphometric and meristic differences between the two groups.

Materials and methods

Sample collection

Samples of white hake were collected during two consecutive bottom trawl surveys (RV *Lady Hammond*) conducted from August 4 to September 24, 1986. Seasonal groundfish surveys of the southern Gulf indicate that at this time of year, white hake from the Strait and Channel groups are most widely distributed and have reached the limits of their summer distribution (Clay 1991). Survey procedures are described in Hurlbut and Clay (1990).

Specimens between 35 and 50 cm (total length) were selected to minimize the effects of allometric growth within the samples. The collected fish were bagged and packed in an undistorted condition in cardboard cartons and then were rapidly frozen (–16.0°C). The resulting collection (Fig. 1*b*) had a preponderance of samples from the northern and southeastern extremes of NAFO Division 4T, although there were some from as far west as the mouth of the St. Lawrence River.

Laboratory methods

Previously, there has been a great deal of confusion regarding the species of *Urophycis* that occurs in the Gulf of St. Lawrence (Leim and Scott 1966; Musick 1969, 1972). This confusion persisted until Musick (1969, 1972) revised the taxonomic characters that permit distinction between *U tenuis* and its morphologically similar congener *Urophycis chuss* (red hake). These characters were examined for each specimen, and any that could not be positively identified as white hake were forwarded to the Atlantic Reference Centre (St. Andrews, N.B.) for confirmation. All specimens were confirmed to be *U tenuis*.

For each specimen, nine meristic and 20 morphometric characters were counted or measured (Table 1; Fig. 2). Most of the morphometric characters were measured as described by Hubbs and Lagler (1958). The exceptions included two of the characters

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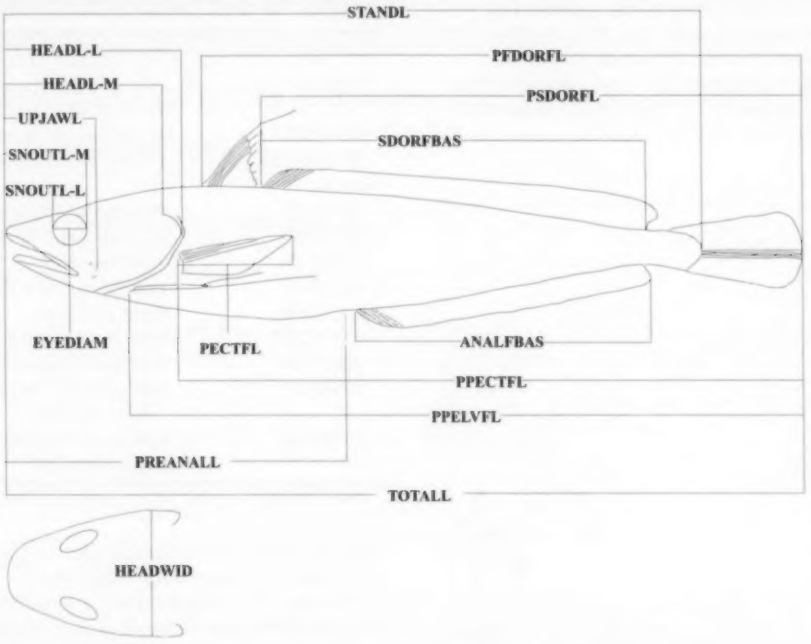
Fig. 2. Morphometric measurements (mm) used in this investigation.



described by Musick (1969, 1972) specifically for morphometric comparisons of *U tenuis* and *U chuss*. These characters are differentiated from analogous characters described by Hubbs and Lagler (1958) by use of the extensions -M or -L in the character names.

Three different sizes of calipers were used for the morphometric measurements. SNOUTL-L, SNOUTL-M, EYEDIAM, HEADL-L, HEADL-M, HEADWID, UPJAWL, and PECTFL, which were <30 cm long for all of the specimens, were measured to the nearest 0.1 mm with vernier callipers. SDORFBAS, ANALFBAS, PREANALL, PFDORFL, PSDORFL, PPECTFL, and PPELVFL tended to be >30 cm and were measured to the nearest millimetre with modified callipers constructed from a meter stick with sliding needle points. OTOLHSL and OTORHSL were measured to the nearest 0.01 mm with dial vernier callipers. TOTALL and STANDL were measured to the nearest millimetre on a measuring board. PECTBGIR was measured with a piece of nylon twine to the nearest millimetre.

Meristic counts were made from X-ray images. The left pectoral fin and first right gill arch were excised and X-rayed separately from the remainder of the carcass. All vertebrae with hemal arches or spines were counted as abdominal vertebrae, and the total vertebra counts excluded the urostyle.

Statistical analysis

Analyses were performed using Statistical Analysis System software (SAS Institute Inc. 1985). Due to the distributional differences between morphometric and meristic characters and because morphometric characters are size dependent (Sokal and Rohlf 1981), the two types of characters were analyzed separately. Morphometric measurements were transformed to common logarithms. Because sexual dimorphism with respect to growth in white hake (Clay 1987) could result in greater variations in morphology than may be attributable to geographic variation between populations, we used ANCOVA, with TOTALL as the covariate, to test for differences in shape between males and females. We also used ANOVA to test for differences between the sexes in the meristic characters because it has been demonstrated that sexual dimorphism may also be manifest in the expression of meristic characters (Kirpichnikov 1981; Lindsey 1988). Sex and its interaction with area were included in the ANCOVA and ANOVA models.

The morphometric characters were standardized to the overall mean total length before discriminant function analyses using pooled within-group slopes determined by ANCOVA and the following formula:

$$\text{ADJCHAR} = \text{ORGCHAR} - [\text{SLOPE} \times (\text{TOTALL} - \text{MEANTOT})]$$

where ADJCHAR is the value of the size-adjusted character, ORGCHAR is the original value of the character, SLOPE is the pooled within-group slope of the respective characters, TOTALL is the total length of each fish, and MEANTOT is the overall mean total length for each sex (43.9 and 42.2 cm for females and males, respectively).

We confirmed that the slopes did not differ significantly among the two groups by comparing models with a constant slope over all groups with those with separate slopes within each group. In all cases, correlation coefficients differed between the two models by <5%, confirming that there were no significant differences in allometric relationships between the areas. A wide variety of statistical procedures, in addition to pooled within-group slopes, have been used to adjust for the effect of size on shape in stock identification studies using morphometrics, but there is currently no clear consensus as to which procedure is superior (i.e., Reist 1985; Claytor and MacCrimmon 1986). An alternative approach is to use the overall regression slope (i.e., the slope over all the groups combined). To confirm that the results were not contingent on the procedure used for size adjustment, we also performed the analysis using characters adjusted with overall regression slopes. This analysis, not presented here, led to the same conclusions reached using characters adjusted with pooled within-group slopes.

Discriminant function analyses contrasted white hake sampled from depths ≤100 m (Strait component) with those from depths >200 m (Channel component). Samples from depths between 100

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Table 2. Morphometric characters (means ± SD) of female and male white hake standardized to the overall mean total length (43.9 and 42.2 cm for females and males, respectively) using pooled within-group regression slopes and results of ANCOVA for sexual dimorphism in morphometric characters.

	Sample						
	Strait		Channel		*p* value		
Morphometric character	Females (n = 92)	Males (n = 167)	Females (n = 171)	Males (n = 169)	Sex effect	Area effect	Sex × area interaction
STANDL	384.6±2.6	369.6±2.6	384.7±2.6	368.9±2.3	0.8462	0.1944	0.0742
SNOUTL-L	23.9±1.8	22.1±1.8	24.3±1.8	22.6±1.4	0.0001	0.0006	0.6387
SNOUTL-M	42.2±2.4	40.5±2.4	44.7±2.5	43.1±2.0	0.5161	0.0001	0.4352
EYEDIAM	16.0±0.9	15.9±1.0	16.3±1.1	16.3±1.3	0.0001	0.0021	0.5479
HEADL-L	96.7±3.1	91.1±3.1	97.7±3.0	92.4±2.5	0.0001	0.0001	0.5685
HEADL-M	91.9±3.0	86.7±3.2	93.1±3.0	88.0±2.6	0.0001	0.0001	0.8796
HEADWID	56.1±5.3	53.6±3.9	55.2±5.8	51.8±5.0	0.9155	0.0009	0.2419
UPJAWL	42.9±3.2	40.5±3.0	42.3±3.1	39.9±2.8	0.0138	0.0293	0.7329
PECTFL	70.2±3.1	67.6±2.7	70.4±2.8	67.3±3.3	0.5411	0.8830	0.2201
SDORFBAS	220.4±4.9	214.0±4.7	219.9±4.8	213.3±4.5	0.0001	0.0862	0.8678
ANALFBAS	171.6±4.7	166.0±4.1	169.6±5.2	165.2±4.5	0.0028	0.0001	0.0991
PREANALL	183.6±4.2	173.7±4.4	183.1±5.3	172.4±4.7	0.0001	0.0422	0.1923
PFDORFL	331.3±2.8	320.0±3.1	330.2±3.5	319.1±3.0	0.0001	0.0001	0.6643
PSDORFL	295.9±4.1	285.8±3.5	294.9±4.2	285.5±3.4	0.0001	0.0284	0.2856
PPECTFL	345.9±3.7	334.2±3.6	346.1±4.1	334.1±3.7	0.0001	0.8292	0.8248
PPELVFL	374.0±5.4	360.9±4.7	375.4±4.7	361.8±4.4	0.0013	0.0061	0.5997
PECTBGIR	211.5±12.3	202.5±13.1	214.7±14.5	202.5±13.9	0.5358	0.1521	0.1724
OTOLHSL	18.8±0.7	18.2±0.6	18.6±0.7	18.2±0.6	0.0739	0.2934	0.1793
OTORHSL	18.7±0.7	18.1±0.7	18.6±0.6	18.2±0.6	0.0650	0.4870	0.0364

and 200 m were not included in the discriminant function analyses (24 females and 43 males; see Fig. 1*b*). A forward, stepwise discriminant function analysis was used to select the characters that best distinguished between the two areas and to evaluate the relative contribution of each character. In the case of highly correlated morphometric characters (i.e., SNOUTL-L and SNOUTL-M and HEADL-L and HEADL-M), the characters with the lowest Wilk's lambda (SNOUTL-L and HEADL-M) were eliminated from subsequent discriminant analyses. We used the kappa (K) statistic to determine the improvement over chance of the classifications derived from the discriminant functions (Titus et al. 1984). A K of zero indicates that no improvement over chance was provided by the discriminant analysis, while a K of 1 occurs only with perfect agreement. To obtain a better estimate of the misclassification rate, the samples were split into two data sets (even versus odd fish numbers). The first data set was then used to derive the discriminant function ("learning" sample) and the second to determine the efficiency of the function ("test" sample) (Pella and Robertson 1979). The samples from depths between 100 and 200 m were classified with discriminant functions derived from the previous classification of samples from the Strait and Channel components.

Results

The ANCOVA for differences in morphometric characters between female and male white hake (Table 2) revealed sexual dimorphism ($p < 0.01$) in 11 of the 19 morphometric characters. A difference ($p < 0.01$) was also detected between female and male white hake for the number of abdominal vertebrae (Table 3). Therefore, the analyses of morphometric and meristic characters were conducted with the sexes separated.

Differences ($p < 0.01$) between the shallow- and deep-water areas were observed for nine of the morphometric characters (Table 2) and three of the meristic characters (Table 3). The interaction between sex and area was not significant for any of the characters except for the number of pectoral fin rays (Tables 2 and 3).

Character correlations were much lower after size adjustment using the pooled within-group regression slopes and averaged 0.017 and 0.009 for females and males, respectively.

Forward, stepwise discriminant function analysis revealed that six of the 19 morphometric characters contributed significantly to the multivariate discrimination between the two areas for female white hake (Table 4). In contrast, eight of the 19 morphometric characters contributed to the multivariate discrimination between the areas for male white hake (Table 4). A greater relative SNOUTL-M in fish sampled from along the Laurentian Channel (Channel component) compared with those from the southern Gulf (Strait component) was the primary character difference for both sexes. Of the remaining morphometric characters selected in the stepwise discriminant function analyses, HEADL-L and PREANALL were the most important, in terms of their discriminating power, for female and male white hake, respectively.

Forward, stepwise discriminant function analysis applied to the meristic data resulted in four characters contributing to the discriminant functions for females and two characters for males (Table 4). PECTFR was the primary character difference between female white hake of Strait and Channel origin (Channel fish > Strait fish), and CERRAK was the

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Table 3. Meristic characters (means ± SD) of female and male white hake and results of ANOVA for sexual dimorphism in meristic characters.

	Sample						
	Strait		Channel		*p* value		
Meristic character	Females (n = 92)	Males (n = 167)	Females (n = 171)	Males (n = 169)	Sex effect	Area effect	Sex × area interaction
FDORFR	9.6±0.7	9.4±0.7	9.4±0.7	9.4±0.8	0.1959	0.2250	0.1953
SDORFR	53.9±1.9	54.2±1.9	54.5±2.0	54.3±1.9	0.7467	0.0464	0.2348
CAUDFR	35.0±1.1	35.0±1.1	35.3±1.1	35.0±1.0	0.1700	0.0680	0.1330
ANALFR	47.8±1.6	47.9±1.8	47.6±1.8	47.9±1.8	0.1835	0.2869	0.4473
PECTFR	16.2±0.8	16.3±0.6	16.6±0.7	16.3±0.8	0.0437	0.0001	0.0023
TOTVERT	48.7±0.6	48.7±0.6	48.8±0.6	48.8±0.6	0.7504	0.3497	0.6677
ABDVERT	15.9±0.4	15.8±0.5	16.1±0.5	15.9±0.4	0.0092	0.0001	0.1584
CERRAK	13.0±0.9	12.9±0.8	13.4±1.0	13.3±0.8	0.2701	0.0001	0.9379
EPIRAK	2.1±0.3	2.1±0.2	2.1±0.3	2.1±0.3	0.5315	0.1490	0.3745

Table 4. Results of forward stepwise and canonical discriminant function analyses using morphometric and meristic characters.

Characters	Sex	Total no.	Characters selected in forward stepwise discriminant function analysis	Standardized coefficients of first canonical variable	Average % correctly classified	K statistic
Morphometric	Female	239	SNOUTL-M	−1.5423	81.30	0.59
			HEADL-L	0.6833		
			UPJAWL	0.4986		
			PECTBGIR	−0.3626		
			PREANALL	0.5776		
			ANALFBAS	0.4654		
	Male	293	SNOUTL-M	−1.4113	83.57	0.66
			HEADL-L	0.2418		
			PREANALL	0.8592		
			UPJAWL	0.5603		
			PECTBGIR	−0.2675		
			ANALFBAS	0.5083		
			SDORFBAS	−0.2492		
			PSDORFL	0.0917		
Meristic	Female	239	PECTFR	0.5876	62.67	0.24
			ABDVERT	0.4458		
			CAUDFR	0.3697		
			CERRAK	0.3824		
	Male	293	CERRAK	0.8628	56.31	0.16
			EPIRAK	0.4732		

primary character difference between male white hake (Channel fish > Strait fish).

A posteriori classifications of white hake into the two areas using discriminant functions derived from morphometric characters were 81% correct for females and 84% correct for males (Table 4). The K statistics indicate that these classifications were significantly better than chance alone (0.59 and 0.66 for females and males, respectively). In contrast, a posteriori classifications using discriminant functions derived from meristic variables were considerably less successful in their ability to correctly classify samples (63 and 56% correct for females and males, respectively; Table 4). These classifications were only marginally better than chance alone (K = 0.24 and 0.16, respectively).

There was considerably more overlap in the distributions of first canonical variable scores with the meristic discriminant functions (Fig. 3*b*) than with the morphometric discriminant functions (Fig. 3*a*). The majority of the specimens that were misclassified by the morphometric discriminant functions (77% of the females and 60% of the males) were sampled from stations near the 200-m depth contour (Fig. 4). These misclassified specimens represent 19% of all of the fish (both sexes) that were sampled from this area.

The test samples could not be efficiently classified with either of the meristic discriminant functions (i.e., males or females). The observed percentages of correct classification (63% for females and 53% for males) were only slightly better than would have been obtained by chance alone (K = 0.18 and 0.08, respectively; Table 5). In contrast, morphometric discriminant functions, derived from learning sam-

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Fig. 3. Mean first canonical variable scores for the discriminant function analyses of (*a*) morphometric and (*b*) meristic characters of female and male white hake.

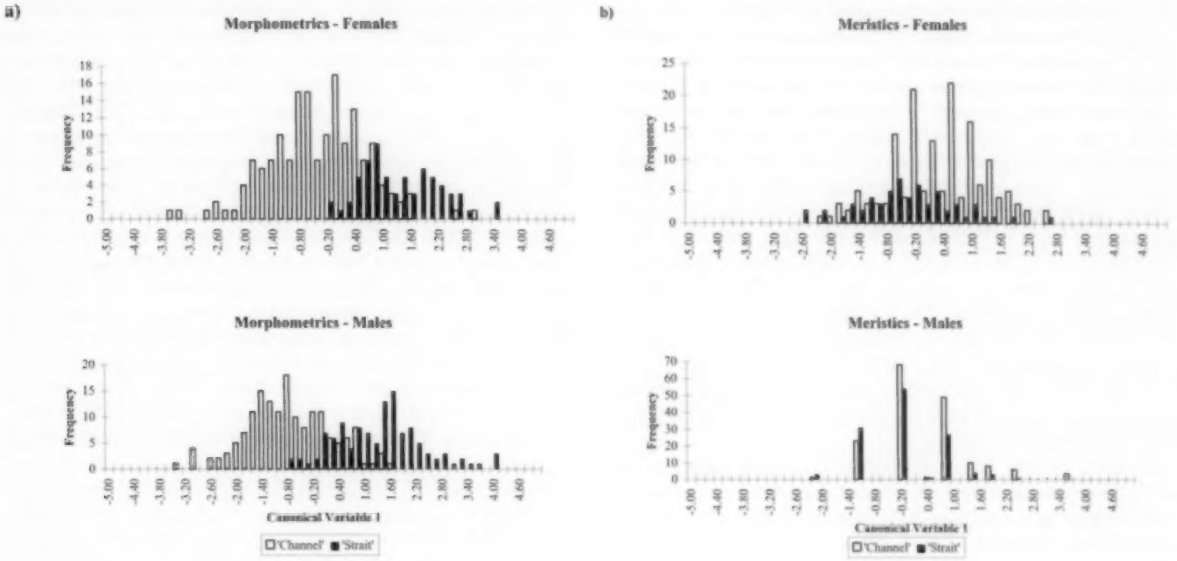
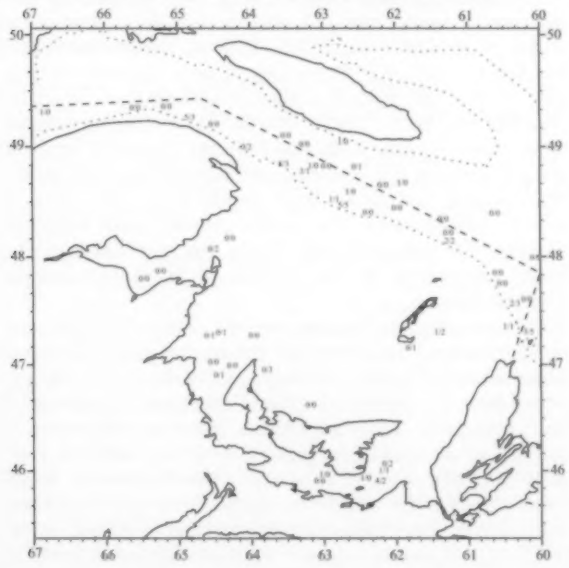


Fig. 4. Number and positions of female and male white hake that were misclassified by the morphometric discriminant functions. The first number at each location represents the number of females and the second the number of males.



ples, were able to correctly classify 82 and 84% of the females and males, respectively, from the test samples. These classifications were significantly higher than chance alone (K = 0.58 and 0.68, respectively; Table 5).

When the samples from depths between 100 and 200 m were classified with discriminant functions derived from the classification of samples from the Strait and Channel components, the majority (59%) were classified as Strait component.

Table 5. Results of the classification of test samples using discriminant function rules derived from learning samples.

Characters	Sex	No. in test data set	Average % correctly classified	K statistic
Morphometric	Female	113	82.30	0.58
	Male	143	83.92	0.68
Meristic	Female	106	63.21	0.18
	Male	150	53.33	0.08

Discussion

This study demonstrates that there is considerable morphological divergence between white hake from the southern Gulf (depths ≤100 m), principally the Northumberland Strait area, and white hake from along the Laurentian Channel (depths >200 m). The best statistical classification of these populations was obtained with morphometric characters, and meristic characters provided limited but congruent evidence for separation. If a "stock" is considered to be an intraspecific group of individuals with recognizable geographic boundaries that exhibit unique phenotypic or genotypic attributes, then based on these results, white hake from the Strait and Channel areas should be regarded as separate stocks. This conclusion is consistent with the results of previous analyses of the distribution of catches of white hake during seasonal and annual surveys of the southern Gulf (Koeller and LeGresley 1981; Clay and Hurlbut 1989; Clay 1991; Morin and Hurlbut 1994) and with the results of a

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limited tagging study conducted off eastern Prince Edward Island (Kohler 1971). It should be noted that no fish from the Channel component were tagged in Kohler's study.

The ability of the morphometric discriminant functions to correctly classify individuals in this investigation compares favourably with the results obtained in discriminant function analyses of other species. In this study, 81% of the female white hake were correctly classified into one of two areas using six morphometric characters, and 84% of the male white hake were correctly classified using eight morphometric characters. Roby et al. (1991) obtained classifications that ranged from 8 to 70% correct for eight groups of capelin (*Mallotus villosus*) using four morphometric characters. Striped bass (*Morone saxatilis*) from two different stocks were correctly classified in 88% of the cases using a reduced set of four truss characters (Waldman et al. 1997). Seventy to 90% of female and male hoki (*Macruronus novaezelandiae*) were correctly assigned to two spawning areas using four and five morphometric characters, respectively (Livingston and Schofield 1996).

Almeida (1987) noted that when the stock structure of nonanadromous marine fish species has been investigated with discriminant analysis (i.e., capelin (Sharp et al. 1978) and summer flounder (*Paralichthys dentatus*) (Wilk et al. 1980)), the percentages of correct classification have generally been lower than for anadromous species. Using a morphometric discriminant function to classify spawning and postspawning silver hake (*Merluccius bilinearis*) from the New England – mid-Atlantic area, Almeida (1987) obtained 76 and 80% correct classification for females and males, respectively (compare this with the morphometric discriminant functions for this investigation, which yielded 81 and 84% correct classification for females and males, respectively; Table 4).

In this study, the meristic discriminant functions correctly classified 63% of the females and 56% of the males, but these classifications were only marginally better than chance alone (Table 4). When Sharp et al. (1978) used meristic discriminant functions to classify capelin from several northwest Atlantic populations, the classifications obtained were only 20 and 27% correct for females and males, respectively. Sharp et al. (1978) and Misra and Carscadden (1987) concluded that meristic characters offered little potential for identification of capelin stocks in Canadian Atlantic waters. In contrast, Shepherd (1991) found that meristic characters provided greater separation between geographic areas than did morphometrics for black sea bass (*Centropristis striata*).

The results of this study are consistent with the findings of Waldman et al. (1997), who compared the performance of six different stock identification approaches (i.e., discriminant analysis of morphometric and meristic data, logistic regression analysis of combined morphometric and meristic data, discriminant analysis of scale shape features, discriminant analysis of immunoassay data, and mixed-stock analysis of mitochondrial DNA) on the same samples of striped bass and concluded that morphometrics-based approaches represent the best phenotypic method.

If differences in phenotypic and (or) genotypic characteristics are accepted as a basis for delineating stocks, then reasons for the occurrence of the phenotypic differences observed between the Strait and Channel stocks should be postulated. A moderately high degree of reproductive isolation is necessary for the formation and maintenance of discrete stocks of fish. This reproductive isolation can be developed through spawning site imprinting and homing, two behavioural mechanisms that are apparently very common in fish (Horrall 1981). Reproductive isolation of the Strait and Channel stocks of white hake probably involves different spawning locations as well as different spawning times. In the Northumberland Strait, the spawning season lasts from June until September, with the peak usually occurring in mid-June (Nepszy 1968). Information on the spawning of white hake from the Channel stock is largely conjectured because this deepwater habitat has not been thoroughly sampled. Markle et al. (1982) observed that spent (i.e., recently spawned) fish were caught in the Laurentian Channel in May and they postulated that spawning probably occurs in late winter – early spring. If this is the case, and if the Laurentian Channel is the location where white hake from the Strait and Channel stocks overwinter, as Clay's (1991) analysis indicated, then the two groups must segregate (either spatially or temporally) during this time in order for the Channel stock to reproduce in isolation. If samples of spawning fish could be obtained from this area, this hypothesis could be tested by conducting a discriminant analysis of their morphometric characters to determine their stock affiliation.

Snout length will likely be an important morphometric character for distinguishing between stocks of white hake. Morphometrics of the head and body depth have been consistently reported among the most important characters for describing geographic trends among fish populations (i.e., the parr of ouananiche and Atlantic salmon (*Salmo salar*) (Riley et al. 1989), anglerfish (*Lophius vomerinus*) (Leslie and Grant 1990), Pacific herring (*Clupea pallasi*) (Schweigert 1990), and orange roughy (*Hoplostethus atlanticus*) (Haddon and Willis 1995)). Martin (1949), rearing rainbow trout (*Oncorhynchus mykiss*) at different controlled temperatures, found that fish reared at cool temperatures had greater head lengths and lesser body depths than fish reared at warmer temperatures. The greater relative snout length of Channel fish compared with Strait fish may reflect cooler water temperatures in the habitats of larval and juvenile Channel fish.

The present study demonstrates the utility of morphometric discriminant functions for defining regional stocks of white hake, but further verification of the stock structure reported here is required. Pepin and Carr (1992) recommended that attempts to define populations based on differences in meristic or morphological features must be verified by genetic evidence in order to confirm that the former differences reflect some degree of reproductive isolation rather than simply environmental distinctiveness. Elliott et al. (1998) found genetic evidence for depth-separated stocks of deepwater spikey oreo (*Neocyttus rhomboidalis*) in Australasian waters. Tagging of white hake from the Strait and Channel components would provide additional information on their stock structure and on the composition of mixed concentrations.

White hake in NAFO Division 4T have been assessed and managed as a single stock. This study suggests that this management unit is not appropriate because of the occur-

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rence of two stocks in it, during the summer and early fall, when there has historically been an important directed fishery for this species. From a fishery management perspective, subdividing the NAFO Division 4T management unit into two zones (≤ 100 and >200 m) would create the problem of how to apportion catches from depths between 100 and 200 m. Since the majority (59%) of the samples in this study from depths between 100 and 200 m were classified as Strait stock, the most tractable approach to this problem would be to subdivide NAFO Division 4T into two zones (≤ 200 and >200 m). An additional fishery management problem occurs when fish from the Strait stock migrate to overwinter along the Laurentian Channel, conceivably outside NAFO Division 4T, when they would become vulnerable to harvest with fish from the Channel stock. In this case, morphometric discriminant functions could be used to apportion catches in the mixture to their respective stocks.

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References

Almeida, F.P. 1987. Stock definition of silver hake in the New England – Middle Atlantic area. N. Am. J. Fish. Manage. **7**: 169–186.

Clay, D. 1987. Assessment of Gulf white hake from NAFO Division 4T in 1987 (including an investigation of otolith size to fish length relationships). Can. Atl. Fish. Sci. Adv. Comm. Res. Doc. 87/81.

Clay, D. 1991. Seasonal distribution of demersal fish (Osteichthyes) and skates (Chondrichthyes) in the southeastern Gulf of St. Lawrence. *In* The Gulf of St. Lawrence: small ocean or big estuary? *Edited by* J.-C. Therriault. Can. Spec. Publ. Fish. Aquat. Sci. No. 113. pp. 241–259.

Clay, D., and Hurlbut, T. 1989. Assessment of Gulf white hake from NAFO Division 4T in 1989 (including an investigation of their distribution in the southern Gulf of St. Lawrence). Can. Atl. Fish. Sci. Adv. Comm. Res. Doc. 89/52.

Claytor, R.R., and MacCrimmon, H.R. 1986. Partitioning size from morphometric data: a comparison of five statistical procedures used in fisheries stock identification research. Can. Tech. Rep. Fish. Aquat. Sci. No. 1531.

Elliott, N.G., Lowry, P.S., Grewe, P.M., Innes, B.H., Yearsley, G.K., and Ward, R.D. 1998. Genetic evidence for depth- and spatially separated stocks of the deepwater spikey oreo in Australasian waters. J. Fish Biol. **52**: 796–816.

Haddon, M., and Willis, T.J. 1995. Morphometric and meristic comparison of orange roughy (*Hoplostethus atlanticus*: Trachichthyidae) from the Puysegur Bank and Lord Howe Rise, New Zealand, and its implications for stock structure. Mar. Biol. **123**: 19–27.

Horrall, R.M. 1981. Behavioural stock-isolating mechanisms in Great Lakes fishes with special reference to homing and site imprinting. Can. J. Fish. Aquat. Sci. **38**: 1481–1496.

Hubbs, C.L., and Lagler, K.F. 1958. Fishes of the Great Lakes region. University of Michigan Press, Ann Arbor, Mich.

Hurlbut, T., and Clay, D. 1990. Protocols for research vessel cruises within the Gulf Region (demersal fish) (1970–1987). Can. MS Rep. Fish. Aquat. Sci. No. 2082.

Kirpichnikov, V.S. 1981. Genetic bases of fish selection. Springer-Verlag, New York.

Koeller, P.A., and LeGresley, M. 1981. Abundance and distribution of finfish and squid from *E.E. Prince* trawl surveys in the southern Gulf of St. Lawrence, 1970–79. Can. Tech. Rep. Fish. Aquat. Sci. No. 1028.

Kohler, A.C. 1971. Tagging of white hake, *Urophycis tenuis* Mitchill, in the southern Gulf of St. Lawrence. Int. Comm. Northwest Atl. Fish. Res. Bull. **8**: 21–25.

Leim, A.H., and Scott, W.B. 1966. Fishes of the Atlantic coast of Canada. Bull. Fish. Res. Board Can. No. 155.

Leslie, R.W., and Grant, W.S. 1990. Lack of congruence between genetic and morphological stock structure of the Southern African anglerfish *Lophius vomerinus*. S. Afr. J. Mar. Sci. **9**: 379–398.

Lindsey, C.C. 1988. Factors controlling meristic variation. *In* Fish physiology. Vol. XIB. *Edited by* W.S. Hoar and D.J. Randall. Academic Press, New York. pp. 197–274.

Livingston, M.E., and Schofield, K.A. 1996. Stock discrimination of hoki (*Macruronus novaezelandiae*) in New Zealand waters using morphometrics. N.Z. J. Mar. Freshwater Res. **30**: 197–208.

Markle, D.F., Methven, D.A., and Coates-Markle, L.J. 1982. Aspects of spatial and temporal cooccurrence in the life history stages of the sibling hakes, *Urophycis chuss* (Walbaum, 1792) and *Urophycis tenuis* (Mitchill, 1815) (Pisces:Gadidae). Can. J. Zool. **60**: 2057–2078.

Martin, W.R. 1949. The mechanics of environmental control of body form in fishes. Ont. Fish. Res. Lab. **70**: 5–76.

Misra, R.K., and Carscadden, J.E. 1987. A multivariate analysis of morphometrics to detect differences in populations of capelin (*Mallotus villosus*). J. Cons. Cons. Int. Explor. Mer, **43**: 99–106.

Morin, R., and Hurlbut, T. 1994. Distribution of witch *(Glyptocephalus cynoglossus* L.) and white hake (*Urophycis tenuis* M.) in the Gulf of St. Lawrence in relation to management units. DFO Atl. Fish. Res. Doc. 94/90.

Musick, J.A. 1969. The comparative biology of two American Atlantic hakes, *Urophycis chuss* and *U. tenuis* (Pisces, Gadidae). Ph.D. thesis, Harvard University, Cambridge, Mass.

Musick, J.A. 1972. A meristic and morphometric comparison of the hakes, *Urophycis chuss* and *U. tenuis* (Pisces, Gadidae). Fish. Bull. U.S. **71**: 279–488.

Musick, J.A. 1974. Seasonal distribution of sibling hakes, *Urophycis chuss* and *U. tenuis* (Pisces, Gadidae) in New England. Fish. Bull. U.S. **72**: 481–495.

Nepszy, S.J. 1968. On the biology of the hake (*Urophycis tenuis* Mitchill) in the southern Gulf of St. Lawrence. M.Sc. thesis, McGill University, Montreal, Que.

Parsons, L.S. 1993. Management of marine fisheries in Canada. Can. Bull. Fish. Aquat. Sci. No. 225.

Pella, J.J., and Robertson, T.L. 1979. Assessment of composition of stock mixtures. Fish. Bull. U.S. **77**: 387–398.

Pepin, P., and Carr, S.M. 1992. Morphological, meristic, and genetic analysis of stock structure in juvenile Atlantic cod (*Gadus morhua*) from the Newfoundland shelf. Can. J. Fish. Aquat. Sci. **50**: 1924–1933.

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Reist, J.D. 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. Can. J. Zool. **63**: 1429–1439.

Riley, S.C., Power, G., and Ihssen, P.E. 1989. Meristic and morphometric variation in parr of ouananiche and anadromous Atlantic salmon from rivers along the north shore of the Gulf of St. Lawrence. Trans. Am. Fish. Soc. **118**: 515–522.

Roby, D., Lambert, J.D., and Sévigny, J.M. 1991. Morphometric and electrophoretic approaches to discrimination of capelin (*Mallotus villosus*) populations in the Estuary and Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **48**: 2040–2050.

SAS Institute Inc. 1985. SAS user's guide, statistics. SAS Institute Inc., Cary, N.C.

Schweigert, J.F. 1990. Comparison of morphometric and meristic data against truss networks for describing Pacific herring stocks. *In* Fish-marking techniques. *Edited by* N.C. Parker, A.E. Giorgi, R.C. Heidinger, D.B. Jester, E.D. Prince, and G.A. Winans. Am. Fish. Soc. Symp. **7**: 47–62.

Sharp, J.C., Able, K.W., Leggett, W.C., and Carscadden, J.E. 1978. Utility of meristic and morphometric characters for identification of capelin (*Mallotus villosus*) stocks in Canadian Atlantic waters. J. Fish. Res. Board Can. **35**: 124–130.

Shepherd, G. 1991. Meristic and morphometric variation in black sea bass north of Cape Hatteras, North Carolina. N. Am. J. Fish. Manage. **11**: 139–148.

Sokal, R.R., and Rohlf, F.J. 1981. Biometry. 2nd ed. W.H. Freeman and Co., San Francisco, Calif.

Titus, U., Mosher, J.A., and Williams, B.K. 1984. Chance-corrected classification for use in discriminant analysis: ecological applications. Am. Midl. Nat. **111**: 1–7.

Waldman, J.R., Richards, R.A., Schill, W.B., Wirgin, I., and Fabrizio, M.C. 1997. An empirical comparison of stock identification techniques applied to striped bass. Trans. Am. Fish. Soc. **126**: 369–385.

Wilk, S.J., Smith, W.G., Ralph, D.E., and Sibunka, J. 1980. Population structure of summer flounder between New York and Florida based on linear discriminant analysis. Trans. Am. Fish. Soc. **109**: 265–271.

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Outcome of an inshore spawning event by northern Atlantic cod (*Gadus morhua*) at a low stock level

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Abstract: A large spawning aggregation of northern Atlantic cod (*Gadus morhua*) was discovered in a fjord of Trinity Bay, Newfoundland, in April 1995, 3 years after the collapse of this stock and 1 year after the last offshore spawning aggregation was recorded. We hypothesized that spawning by this aggregation would increase the abundance/density of eggs and settled 0-group juveniles above the level of spawning in the bay during recent years. Data from 1995 were compared with ichthyoplankton and postsettlement juvenile surveys conducted in recent years. Egg density was significantly higher in 1995 than the pooled average of two recent years (1991 and 1993). The hypothesized increase in numbers of postsettlement age 0 fish was tested at three different spatial scales: the entire northeast coast of Newfoundland, individual bays, and areas within Trinity Bay. There was no detectable increase in catches of settled age 0 juveniles at any spatial scale. Consequently, while this large spawning aggregation produced locally high egg concentrations, it did not lead to a detectable increase in abundance of newly settled age 0 juveniles in coastal nursery areas. This study did not provide any direct evidence for an increase in recruitment in the inshore region due to the spawning output of locally abundant spawners in Trinity Bay.

Résumé : Chez la morue du Nord (*Gadus morhua*), un important rassemblement de géniteurs a été observé dans un fjord de la baie de la Trinité (Terre-Neuve), en avril 1995, trois ans après l'effondrement de ce stock et un an après l'observation du dernier rassemblement de géniteurs en haute mer. Nous croyons que la reproduction d'un nombre aussi important de géniteurs peut entraîner un accroissement de l'abondance et de la densité des oeufs ainsi que des juvéniles du groupe d'âge 0 établis sur le fond supérieur aux niveaux observés dans la baie au cours des dernières années. Nous avons comparé les données de 1995 aux relevés de l'ichtyoplancton et des juvéniles établis sur le fond réalisés au cours des dernières années. La densité des oeufs était significativement plus élevée en 1995 que la moyenne regroupée de deux années récentes (1991 et 1993). L'augmentation présumée du nombre de juvéniles de la classe d'âge 0 établis sur le fond a été vérifiée aux trois échelles spatiales suivantes : l'ensemble de la côte du nord-est de Terre-Neuve, une série de baies considérées individuellement et différents secteurs de la baie de la Trinité. Aucune augmentation perceptible du nombre de prises de juvéniles d'âge 0 après établissement n'a été observée à aucune de ces échelles. En conséquence, même s'il a produit localement une forte concentration d'oeufs, cet important rassemblement n'a pas entraîné une augmentation détectable de l'abondance des juvéniles de la classe d'âge 0 récemment établis sur le fond dans les zones d'alevinage côtières. Cette étude n'a pas fourni de preuve directe d'une augmentation du recrutement dans la région côtière imputable à la reproduction d'un grand nombre de géniteurs dans la baie de la Trinité.

[Traduit par la Rédaction]

Introduction

Recruitment to commercially important groundfish typically undergoes large annual variation, which often is unexplained despite nearly a century of research. The prevailing theory states that year-class strength is determined during the pelagic (egg and larval) stages when mortality is high due to starvation (Hjort 1914), which may result from variation in physical processes (e.g., Cushing 1975). More recently, many researchers have hypothesized that the recruitment "signal" driven by mortality in the planktonic phase may be modified by variation in postsettlement juvenile mortality (e.g., Sissenwine 1984; Bradford 1992). Houde (1987) and Perry and Neilson (1988) have suggested that, under some circumstances, mortality in the early settled juvenile stage may be just as important as egg and larval mortality. Sissenwine (1984) proposed that variations in postsettlement juvenile mortality may have a greater effect on recruitment than egg and larval mortality due the cumulative effects of the much longer duration of the settled juvenile life stage. Peterman et al. (1988) have concluded that if recruitment variability is to be better understood, research

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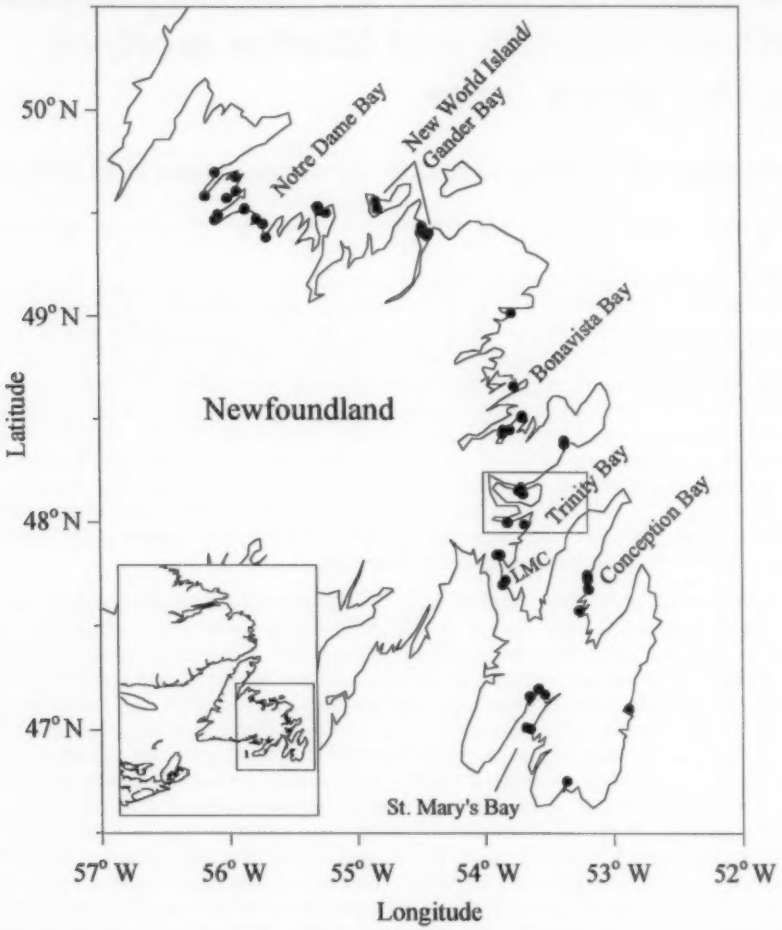
Fig. 1. Map of Newfoundland showing sites from the postsettlement juvenile survey. Each circle represents a beach-seining location.



should be undertaken on all life stages, and especially those closer to age 1. This recommendation has been supported by the results of recent investigations of the dynamics of groundfish during the first year of life (Fromentin et al. 1997; Hüssy et al. 1997).

In recent years, Atlantic cod (*Gadus morhua*) inhabiting NAFO Divisions 2J3KL (known as the northern cod stock) has undergone a dramatic decline in biomass (Hutchings and Myers 1994). As a result, this stock has been under a fishing moratorium since 1992. The spawning biomass has not yet recovered, and stock size remains at low levels (Shelton et al. 1996*a*). The collapse spurred research into the spawning behaviour and early life history of northern cod in an effort to better understand the dynamics of the population. Reports of inshore spawning of northern cod (Hutchings et al. 1993; Smedbol and Wroblewski 1997) led to questions concerning the possible contribution of inshore spawning components to the rate of recovery of the stock. In addition, genetic (Ruzzante et al. 1996) and behavioural (Wroblewski et al. 1996) studies have indicated that these known spawning areas may be occupied by distinct inshore subpopulations.

The importance of estimating the relative influence of inshore spawning on overall northern cod abundance, whether by migrant fish or resident inshore stock components, has increased due to the disappearance of offshore spawning components (Shelton et al. 1996*a*). As a result, the majority of spawning biomass known to exist for this population is now located in the nearshore area of northeast Newfoundland (e.g., Anonymous 1998). This aggregated spatial distribution is favourable for the study of recruitment in early life stages of northern cod because spawning products are more accessible for sampling than when spawning was spread throughout the population's former range.

The Random Island area of Trinity Bay (Figs. 1 and 2) is a known spawning area for cod (Smedbol and Wroblewski 1997). The discovery in Trinity Bay of a relatively large inshore spawning aggregation of cod (16 800 t; Rose 1996) during this period of stock collapse provided a novel opportunity to monitor a single cohort through its early life history. Taggart (1997) has hypothesized that this aggregation may consist of those fish that would normally aggregate offshore in the North Cape region (northern Grand Banks, NAFO Division 3L) during the winter months, but for some reason ceased their usual offshore–inshore seasonal migra-

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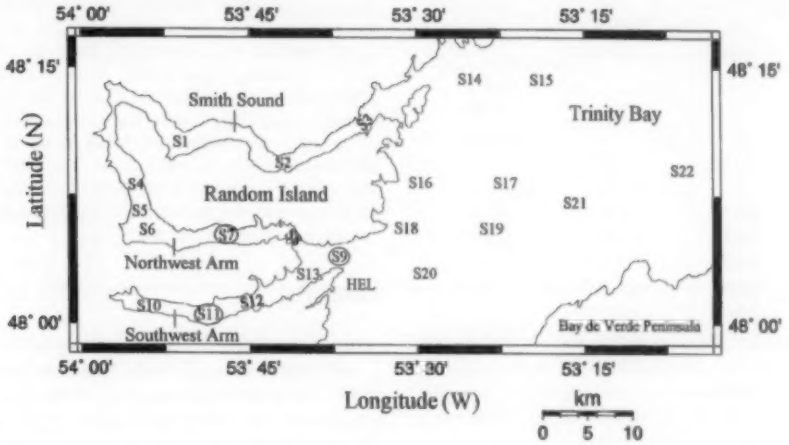
Fig. 2. Map of the Random Island area of Trinity Bay showing the ichthyoplankton survey sites sampled in 1991, 1993, and 1995. HEL is Heart's Ease Ledge, a known spawning area (Smedbol and Wroblewski 1997). The three stations that were sampled during every survey period (S7, S9, and S11) are encircled.



tion and remained inshore. An aggregation of varying size has been detected in this location during the spring of 1996 (171 t) and 1997 (21 000 t) (Anonymous 1998).

We expected that this large spawning aggregation might provide a spawning "signal" that could be readily detected at subsequent life history stages when compared with the usual level of spawning by resident and (or) migrant populations of cod in the bay as measured in recent years. A substantial increase in cod egg density compared with recent years was expected. This pulse in spawning output was expected to pass through the early life stages and result in increased densities of postsettlement age 0 juveniles, although due to cumulative mortality, this increase was predicted to be of lesser magnitude than the expected increase in egg density. Therefore, to test whether the spawning of the aggregation of cod discovered in Smith Sound, Trinity Bay, would lead to a detectable increase in the abundance/density of eggs and postsettlement age 0 juveniles, the results of eggs and settled juvenile abundance surveys in 1995 were compared with surveys undertaken in the area in previous years.

Methods

Ichthyoplankton survey

During June and July 1991, ichthyoplankton samples were collected at 10 stations in Northwest Arm and Southwest Arm of Trinity Bay (Fig. 2). Samples were collected again from these stations during late June – early July 1993. Ichthyoplankton samples were also obtained near a small aggregation of spawning cod found near Heart's Ease Ledge during July 3–7, 1993 (Smedbol and Wroblewski 1997). During June 1995, several of these locations were again sampled, together with additional stations in western Trinity Bay near Random Island. Three stations, S7, S9, and S11, were occupied in each of the above sampling periods (Fig. 2).

Plankton sampling was undertaken with a 1-m ring net with 333-μm mesh throughout. Oblique tows were conducted with elapsed times of 7–25 min and a tow velocity of 0.5–1.0 $m \cdot s^{-1}$ (1–2 kn) with payout and retrieval rates of about 0.5 $m \cdot s^{-1}$. Maximum tow depth was about 80 m. While the depth of individual tows varied, all tows reached depths greater than the mixed layer, the depth of which was determined from CTD casts at each station. Volumes filtered were calculated using flowmeter readings. Plankton samples were fixed in a 4% buffered formalin–seawater solution. Cod eggs were identified to stage of development following the criteria of Page and Frank (1989). Date of spawning of staged cod eggs was backcalculated using the relationship of egg stage duration to incubation temperature reported in table 9 of Page and Frank (1989). Methods for the derivation of temperature for use in this relationship are reported in Smedbol and Wroblewski (1997). Briefly, the temperatures used to estimate the age of eggs represent the mean temperature from the surface to the 0°C isotherm at stations in the Random Island area. In 1991 and 1993, temperatures from a station near station S8 were used (Fig. 2). In 1995, the temperature profile from station S2 was used (Fig. 2), as this station was inside the area occupied by the spawning aggregation.

Early-stage cod eggs cannot be distinguished from early-stage haddock (*Melanogrammus aeglefinus*) and witch flounder (*Glyptocephalus cynoglossus*) eggs (Fahay 1983). Such eggs were termed "chw." Haddock has been very rare in the region since the mid-1960s (Murphy 1995). Witch flounder has recently undergone a dramatic decline and change in distribution (Bowering et al. 1993) and currently is rare in Trinity Bay relative to cod. In addition, no late-stage haddock or witch flounder eggs were collected in the surveys. Thus, all chw eggs were assumed to be cod eggs, even though some unknown but small proportion of the samples may be composed of the other two species.

Survey of demersal age 0 juvenile cod

In recent years, the vast majority of postsettlement age 0 cod have been limited to the inshore area within the population range of northern cod (Anderson and Dalley 1997). Extensive sampling of the shallow, coastal nursery zone, defined as 55 m from shore (Schneider et al. 1997), by a variety of gears indicated that maximum densities of first-year juvenile cod occurred at 4–7 m depth and that cod density decreased with increasing depth/distance offshore to about 30–50 m, the maximum depths sampled (Methven and Schneider 1998). The relationship between abundance and depth offshore was also evident from transects extending from the coast to hundreds of kilometres offshore, which showed highest densities of 0-group cod occurring along the inshore segment of the transects (Dalley and Anderson 1997). Consequently, we used samples from the coastal nursery zone to determine if the large ag-

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Table 1. Cod egg densities (eggs·m^{-3}) at each station and survey period.

Station	June 1991 (day 168)	July 1991 (day 198)	June–July 1993 (days 180–187)	June 1995 (days 171–173)
S1				1.448
S2				0.783
S3				0.387
S4	0.017	0.116	0.104	
S5	0.006		0.096	
S6		0.129	0.445	0.12
S7	0.008	0.01	0.085	1.119
S8	0.011	0.006	0.057	
S9	0.007	0.004	0.036	0.517
S10	0.009	0.007	0.011	
S11	0.025	0.002	0.002	0.115
S12			0.014	
S13	0.116	0.022		
HEL (1)			0.078	0.662
HEL (2)			0.088	
HEL (3)			0.012	
S14				0.37
S15				0.122
S16				0.227
S17				0.266
S18*			0.001	
S19*			0.001	
S20				0.524
S21*			0.001	
S22*			0.038	

Note: Samples marked with an asterisk were excluded from the statistical analyses because in 1993, these stations were outside the region of observed spawning. HEL 1, 2, and 3 represent repeated samples at the same station.

gregation of spawning cod observed in Smith Sound in 1995 increased the coastal abundance of recently settled juvenile cod in subsequent months.

From 1959 to 1964 the Fisheries Research Board of Canada conducted a series of annual surveys along the coast of eastern Newfoundland to assess the abundance of postsettlement juvenile cod (Fig. 1). These surveys were repeated in 1992–1995, with special care taken to ensure that the same sites were sampled in each survey (Schneider et al. 1997). These surveys target nursery areas (≤ 10 m depth) where densities of age 0 individuals greatly exceed those in deeper water (Methven and Schneider 1998). The fishing gear deployed in each survey was a 25-m bottom seine hauled by two people towards shore after being deployed from a small boat. Seine deployment and specifications follow Lear et al. (1980) and Methven and Schneider (1998). Escapement by small cod is low, based on observations by divers (Schneider et al. 1997).

Three tows were conducted at each site in 1992–1995. Two consecutive sets were made at exactly the same location, with an additional set being conducted immediately adjacent to the first two sets. All sets were conducted during daylight independent of tide level, with the first two consecutive sets being conducted usually within 1 h of each other. Sampling was not confined to any particular time during daylight hours.

Fish were measured for standard length (SL, millimetres) in the field. Standard length was used to divide the catch into three length groups (LGs) as defined by clear modes in the catch from several types of fishing gear previously deployed to sample postsettlement juvenile cod in shallow water along the coast of Newfoundland (Methven and Schneider 1998). Of importance in this study was the length group corresponding to age 0 cod (LG0), which consisted of cod < 96 mm SL (Methven and Schneider 1998). The length groups were derived from the aging of a subsample of fish by counting the daily growth increments on the lapillus otolith. For aging methods, see Pinsent and Methven (1997).

It is presently (mid-1990s) difficult to distinguish between recently settled *G. morhua* and *Gadus ogac* (Greenland or rock cod), a morphometrically similar and co-occurring species. Identification of cod was undertaken according to the criteria of Methven and McGowan (1998). In 1992, 1994, and 1995, species identification was carried out in the field. In 1993, *G. morhua* and *G. ogac* were identified after the survey using preserved samples instead of identifying specimens in the field.

Results

Abundance/density of pelagic eggs

All stages of eggs were found in all years. In 1991 and 1993, the oldest eggs (stage IV) were 22 and 17 days old, respectively. In 1995, the oldest eggs in the sample were 16 days old. In all years, most of the eggs were less developmentally advanced than stage IV. For example, in 1995, less than 15% of the eggs collected were classed as stage IV.

Egg densities were generally one order of magnitude greater in 1995 than in 1991 and 1993. Cod egg density within and adjacent to Smith Sound was significantly higher in 1995 than the pooled average of the two previous sampling years (Tables 1 and 2; Fig. 3). Mean densities were greater when all stations were used in the analysis (ANOVA, $F = 33.17$, $p < 0.0002$, $n = 41$; nonnormal error structure, so the p-value was calculated from 5000 data randomizations). The 1995 mean (0.51 egg·m^{-3}) was 14 times greater than the pooled mean of the surveys in 1991 and 1993 (0.04 egg·m^{-3}) (Table 2; Fig. 3). If only the three stations occupied in every sampling period were used, the difference was just short of significant (ANOVA, $F = 3.71$, $p = 0.061$, $n = 12$; p-value calculated from 5000 data randomizations) (Fig. 3). The 1995 mean (0.58 egg·m^{-3}) was 30 times greater than the pooled mean from 1991 and 1993 (0.01 egg·m^{-3}) (Table 2; Fig. 3).

Abundance and distribution of LG0 demersal juvenile cod

Increase in catch rate of LG0 fish was tested at three different spatial scales: the entire northeast coast of Newfoundland, individual bays, and areas within bays. At the coastal scale, no increase in average catch was observed in 1995 over recent years (Fig. 4). The average catch of LG0 fish in 1995 was 13.3 fish·haul^{-1}, close to the densities measured during 1992–1994 (Fig. 4). Ages and hatch dates of a subsample of the LG0 cod are indicated in Table 3.

Increased catches of postsettlement age 0 cod were expected at the scale of individual bays, with greater abundances in Trinity Bay and perhaps Conception Bay in 1995 than in preceding years (1992–1994). Conception Bay is included in the prediction because the prevailing flow is towards Conception Bay, which lies south of Trinity Bay (DeYoung et al. 1993) (Fig. 1). Contrary to expectation, increased catches of LG0 cod were not observed in Trinity or Conception Bay in 1995, relative to preceding years (Fig. 5). In 1995, the observed mean catch was less than the pooled

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Table 2. Comparison of cod egg densities (eggs·m^{-3}) in 1995 and pooled surveys in 1991 and 1993.

	Mean density	Ratio of means, all stations		Mean density, common stations only	Ratio of means, common stations only	
		1995/sample year	1995/pooled years		1995/sample year	1995/pooled years
June 1991 (day 168)	0.025	20.5	14	0.013	45	30
July 1991 (day 198)	0.037	13.9		0.005	117	
June–July 1993 (days 180–187)	0.064	7.97		0.041	14	
June 1995 (days 171–173)	0.512			0.584		

Fig. 3. Mean cod egg density from stations in the Random Island area of Trinity Bay calculated from all stations (circles) and only stations S7, S9, and S11 (squares), which were sampled in every survey period (1991, 1993, and 1995). Error bars represent standard errors.

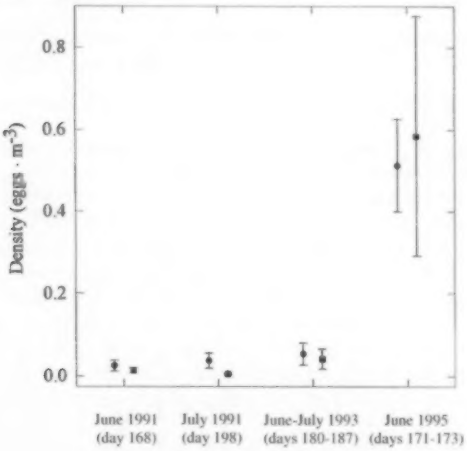
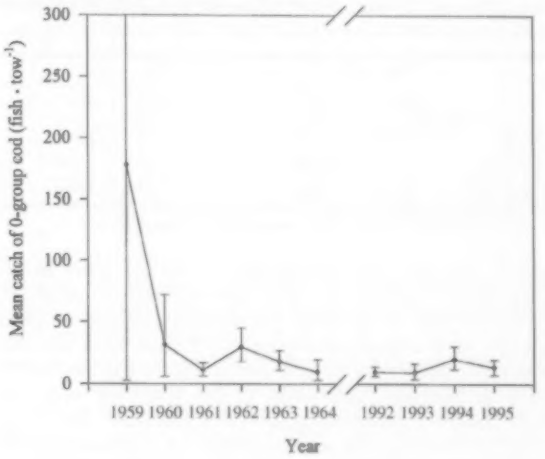


Fig. 4. Mean catch of length group 0 cod from the entire juvenile survey in 1992–1995 compared with historical catches (1959–1964). Error bars represent 95% confidence limits derived from bootstrapping of individual tows.



average catch of the years 1992–1994 (Table 4). There was no evidence of greater numbers of LG0 fish anywhere along the coast in 1995, relative to the previous three years (Fig. 5).

Higher catch rates at the scale of sections of Trinity Bay also were hypothesized, with greater catches in the inner part of Trinity Bay due to lower exchange rates and greater retention in the inner bay. Also, the prevailing drift along the west side of Trinity Bay runs toward the inner bay from Smith Sound (Yao 1986). Increased catch rates within Trinity Bay were not observed in 1995, relative to previous years (Fig. 6). Catches near Smith Sound (Random Island sites) were low in all years. During 1995, catches to the north and south of Random Island matched that observed in the early 1960s (Fig. 6).

Discussion

The spawning of the cod aggregation within Trinity Bay in 1995 resulted in elevated concentrations of eggs relative to recent years. However, this did not result in a detectable increase in the number of settled age 0 juveniles in the region. There are several possible explanations.

Were the postsettlement juvenile survey sites too widely spaced (Fig. 1) to detect local increases in density? The analysis was carried out at three scales of spatial resolution: the coast of eastern Newfoundland, individual bays, and areas within Trinity Bay. A finer scale of resolution was not possible because of the lack of prior measurements at more sites in Trinity Bay. Thus, a more localized increase in abundance would have gone undetected. However, typical scales of horizontal eddy diffusivity acting over a 90-day period (500 m^2·s^{-1}) will diffuse particles over an area of 3900 km^2, with a diameter of 70 km. This is of the same order of magnitude as Trinity Bay. The sample spacing of this survey was sufficient to detect a density increase, unless eggs and larvae drifted in a more cohesive pattern than the surrounding water, leading to settlement in one highly restricted area.

It is possible that the error rate of the postsettlement juvenile survey may have been too high to detect an increase in abundance. The error rate of the survey, as measured by bootstrapped estimates of 95% confidence limits (Figs. 4

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Table 3. Ages (days) of length group 0 cod caught in Little Mosquito Cove (LMC, Fig. 1), Trinity Bay, on October 4, 1995.

Specimen	SL	Count 1	Count 2	Count 3	Hatch date
1 left	56	97	93	90	July 3 (day 184)
1 right		95	91		
2	53	89	95	96	July 3 (day 184)
3	57	101	96	100	June 27 (day 178)
4	59	103	99	93	June 28 (day 179)
5	71	115	124	130	June 3 (day 154)

Note: Count is the number of daily increments (three successive counts). SL, standard length (mm). Estimated hatch date = 277 – mean age (mean count).

Table 4. Mean catch (fish·haul^{-1}) of length group 0 cod in 1995 inside Trinity Bay and outside (all other bays pooled) compared with recent (1992–1994) catches inside and outside Trinity Bay.

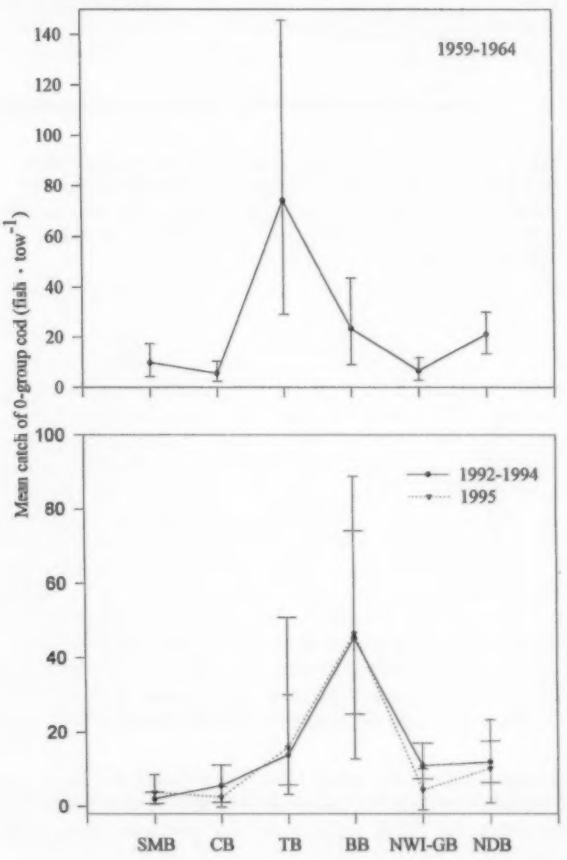
	1992–1994			1995		
	Mean	SD	*n*	Mean	SD	*n*
Inside	21.00	3.964	81	15.96	6.865	27
Outside	14.5	2.004	317	11.53	3.964	81
Combined	17.76	2.221	398	23.75	3.964	108

Note: Combined means have been weighted by sample size.

and 5), was low, but still substantial. The true value of LG0 abundance (Fig. 4) in 1995 lies between 7.1 and 19.4 fish·haul^{-1} 95% of the time. Given the uncertainty of the estimate, what change in density could have been detected? The observed density of LG0 cod in Trinity Bay in 1995 relative to previous years was (15.96 fish·haul^{-1})/(21 fish·haul^{-1}) = 76% compared with (11.53 fish·haul^{-1})/(14.5 fish·haul^{-1}) = 80% outside the bay (Table 4). The minimum detectable increase inside Trinity Bay, at the 5% significance level, was (35.96 fish·haul^{-1})/(21 fish·haul^{-1}) = 171%, based on an analysis of the means in Table 4 (minimum increase to obtain a significant interaction term in a two-way ANOVA). The survey could have detected, with a high degree of confidence, an increase of 171–100 = 71% in density in 1995, relative to previous years. The minimum increase in demersal age 0 juveniles that could have been detected due to the statistical power of the survey was much less than the observed increase of 1400% in egg density.

The presence and spawning of the large aggregation in the Smith Sound area in 1995 may have been an anomalous or relatively rare event and not related to the annual spawning in the bay. Alternatively, this aggregation may not have represented additional spawning biomass, but rather resulted from a patchier distribution of adults from within Trinity Bay. While the origin of these fish is not currently known, such large aggregations have not previously been detected in the area during early spring, either by field studies (Goddard et al. 1994; Wroblewski et al. 1994; Smedbol and Wroblewski 1997) or by sampling for tag and recapture programs (Lear 1984; Taggart et al. 1995). This is despite growing genetic (Ruzzante et al. 1996) and behavioural

Fig. 5. Mean catch of length group 0 cod from St. Mary's Bay to Notre Dame Bay in 1995 compared with the three previous years (1992–1994) and with historical catches (1959–1964) from the same survey sites (note the change in scale). Error bars represent 95% confidence limits derived from bootstrapping of individual tows. Confidence limits for the 1992–1994 means are displayed with wider error bars. SMB, St. Mary's Bay and Southern Shore; CB, Conception Bay; TB, Trinity Bay; BB, Bonavista Bay; NWI-GB, New World Island/Gander Bay; NDB, Notre Dame Bay.



(Wroblewski et al. 1996) evidence for a resident subpopulation in the area. The presence of this particular assemblage of spawners in the bay in 1995 during the early spring may be associated with the recent changes in spatial distribution of northern cod following the collapse of the stock (Kulka et al. 1995; Rose 1996). Aggregations were detected in the area in 1996 and 1997 (Anonymous 1998). While the sudden appearance of a large aggregation along the coast of northeast Newfoundland has not been previously documented, a similar occurrence was recorded in coastal Maine during the 1940s (Ames 1997). Even though the appearance of this spawning aggregation in Trinity Bay may be rare on a local scale, such movements of large schools of adult cod into inshore areas may not be completely anomalous events in this species.

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The Smith Sound aggregation may not have produced enough eggs to result in a detectable increase in density at the demersal juvenile stage. This hypothesis can be addressed by comparing the egg densities measured in Smith Sound with the total possible number of eggs produced by the aggregation and with egg densities recorded in recent years. If we assume that 0.04 egg·m^{-3} represents egg densities in 1991 and 1993 throughout all of Trinity Bay, it is then possible to calculate the factors by which the spawning aggregation could potentially increase egg density.

Given the average mass of 1.6 kg and number of individuals at 1.05×10^7 (Rose 1996), an estimate of the aggregation's total fecundity can be calculated. Average weight was converted to average length using a regression relationship for current stock structure (Shelton et al. 1996*b*):

$$(1) \qquad W = 10^{-5.2106} L^{3.0879}$$

where W is mass (kilograms) and L is length (centimetres). Hence:

$$(2) \qquad L = 10^{\frac{5.2106}{3.0879}} W^{\frac{1}{3.0879}}$$

Fecundity was then calculated using a regression equation provided by May (1967):

$$(3) \qquad F = 0.50 L^{3.42}$$

where F is fecundity (number of eggs) and L is average length (56.7 cm; calculated from eq. 2). Average fecundity was estimated to be 4.96×10^5 eggs. Assuming a 50:50 sex ratio and 70% maturity at a length of 55 cm (May 1967), the total number of eggs spawned by the aggregation was 1.83×10^{12}. If these eggs diffused over the entire area of Trinity Bay (3870 km^2), the resulting density would be 5.36 eggs·m^{-3} in the surface layer, down to the maximum sampling depth of 80 m. This density is 134 times greater than the levels in 1991 and 1993. This calculation does not account for losses from the patch, such as nonfertilization or mortality of eggs.

The density measured in the sample area during 1995 (Fig. 1) was 0.51 egg·m^{-3} (Table 2). This "patch" of eggs can be spread throughout Trinity Bay to calculate a simple overall concentration of eggs. If a border is drawn around the survey stations, the enclosed area covered by the survey is 405 km^2. Thus, the calculated overall egg concentration from spreading out the patch is

$$(4) \quad (\text{Egg density})_{\text{overall}} = (\text{Egg density})_{\text{survey}} \frac{(\text{Area}_{\text{survey}})}{(\text{Area}_{\text{Trinity Bay}})}$$

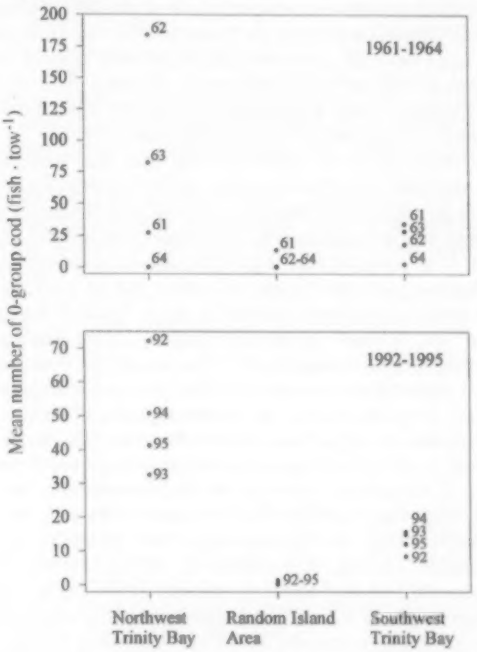
giving a density of 0.05 egg·m^{-3} as follows:

$$(5) \quad (0.51 \text{ egg} \cdot \text{m}^{-3}) \frac{(405 \text{ km}^2)}{(3870 \text{ km}^2)} = 0.05 \text{ egg} \cdot \text{m}^{-3}$$

Egg densities in the study area measured in years prior to the detection of the large aggregation (1991 and 1993) averaged 0.04 egg·m^{-3} (Smedbol and Wroblewski 1997). This pooled mean density can be used as a comparison to evaluate the potential impact of the 1995 aggregation on overall egg densities in Trinity Bay. The total egg output from spawning females, extrapolated to all of Trinity Bay (5.36 eggs·m^{-3}), is 134 times greater than the pooled mean density in 1991 and 1993. Average egg density (0.51 egg·m^{-3}) measured during the Random Island survey in 1995 was 12 times the pooled mean density from the 1991 and 1993 surveys, but when these survey densities are diffused throughout Trinity Bay, the calculated density (0.05 egg·m^{-3}) is close to the pooled average of 1991 and 1993. Thus, if the egg patch produced by the spawning aggregation in 1995 is allowed to spread throughout the bay, the resultant density is not much greater than levels measured in 1991 and 1993. The egg patch had enough time to diffuse throughout the bay, which would remove any potential signal of increased numbers of settled age 0 juveniles due to the assumed increase in egg production over recent years from the presence of the aggregation in Smith Sound, Trinity Bay. From these calculations, we conclude that in 1995, (*i*) egg production was great enough to elevate egg densities in Trinity Bay and (*ii*) the realized egg density from the aggregation of spawners in and around Smith Sound was not enough to elevate egg densities in Trinity Bay.

Trinity Bay is a semienclosed embayment that is free to undergo exchange with the coastal ocean. Conditions can be temporally variable due to environmental influences such as wind forcing. The eggs may thus have been advected out of Trinity Bay by sustained southwesterly winds experienced in June 1995. Direct modelling studies of egg and (or) larval advection do not exist for Trinity Bay, but DeYoung et al. (1994) have developed an advection–diffusion model of neighbouring Conception Bay (Fig. 1) to study the dispersion of capelin (*Mallotus villosus*) larvae. Conception Bay is

Fig. 6. Mean catch of length group 0 cod within Trinity Bay in 1995 compared with recent catches (1992–1994) and with historical catches (1961–1964) from the same survey sites (note the change in scale).



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generally equivalent in size, shape, and orientation to Trinity Bay (Fig. 1). In their model, DeYoung et al. (1994) calculated residence times ranging from 20 to 40 days for larvae originating near the inner, southwest portion of the bay. Using these results for comparison, it is possible that a substantial portion of the egg patch sampled in the 1995 survey may have been advected out of Trinity Bay prior to metamorphosis and juvenile settlement, diffusing over a much larger coastal area. Postsettlement juvenile catches downstream in Conception Bay were very low (Fig. 5).

An aggregation of spawning fish that may comprise a large component of the remaining spawning biomass did not lead to any detectable increase in density of demersal age 0 juvenile fish in traditional nursery areas (the inshore coastal zone). It is possible that high mortality of eggs due to low developmental temperatures may have led to poor recruitment to the postsettlement juvenile stage. Most of the aggregation was located in subzero waters (–1°C) during the spawning period (unpublished data) in 1995. Pepin et al. (1997) reported an increase in cumulative mortality during the egg stage in relation to decreasing temperature during development in eggs from the northern cod stock. In that laboratory study, cod eggs exposed to subzero temperatures during development exhibited the highest mortality prior to hatch. High egg mortality due to exposure to subzero temperatures during development has been proposed as one mechanism limiting recruitment to the cod stock in the northern Gulf of St. Lawrence (Ouellet 1997). Also, with an average length of 55 cm, the majority of the female spawners in the aggregation may have been spawning for only the first or second time (see review by Taggart et al. 1994). Small, first-time spawning females breed for a shorter period, have smaller eggs, and have lower relative fecundity, fertilization, and hatching rates relative to repeat spawners (Kjesbu 1994; Trippel 1998).

Very few cod larvae were captured during the egg survey. Gear avoidance may have been an important factor. Also, the offspring from the Smith Sound spawning aggregation may have been exposed to mortality rates during the larval and pelagic juvenile phases, resulting in the low densities of postsettlement juveniles observed in 1995.

The presence of spawners on a local scale, even in relatively high abundance, does not automatically result in elevated levels of local recruitment to the settled juvenile life stage. The high egg densities produced by the spawning aggregation did not lead to higher catches of postsettlement age 0 cod relative to previous years. Why this spawning "signal" did not translate through to the postsettlement stage is unclear. This study highlights the need for recruitment studies of marine fishes to be undertaken on all early life stages including the egg, larval, pelagic juvenile, and postsettlement juvenile phases. One apparent implication of the results is that inshore spawning by resident and migratory stock components may not be solely capable of restocking the large area that the stock complex once inhabited. However, it must be noted that the study location is only one area along the northeast coast of Newfoundland.

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References

Ames, E.P. 1997. Cod and haddock spawning grounds in the Gulf of Maine. Island Institute, Rockland, Me.

Anderson, J.T., and Dalley, E.L. 1997. Spawning and year-class strength of northern cod (*Gadus morhua*) as measured by pelagic juvenile cod surveys, 1991–1994. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 158–167.

Anonymous. 1998. DFO science stock status report for northern cod A2-01. Department of Fisheries and Oceans, Science Branch, St. John's, Nfld.

Bowering, W.R., Power, D., and Brodie, W.B. 1993. The status of the witch flounder stock in Division 2J, 3K, and 3L. DFO Atl. Fish. Res. Doc. 93/49.

Bradford, M.J. 1992. Precision of recruitment predictions from early life stages of marine fishes. Fish. Bull. U.S. **90**: 439–453.

Cushing, D.H. 1975. Marine ecology and fisheries. Cambridge University Press, New York.

Dalley, E.L., and Anderson, J.T. 1997. Age-dependent distribution of demersal juvenile Atlantic cod (*Gadus morhua*) in inshore/offshore northeast Newfoundland. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 168–176.

DeYoung, B., Otterson, T., and Greatbatch, R.J. 1993. The local and non-local response of Conception Bay to wind forcing. J. Phys. Oceanogr. **23**: 2636–2651.

DeYoung, B., Anderson, J., Greatbatch, R.J., and Vary, P. 1994. Advection–diffusion modelling of larval capelin (*Mallotus villosus*) dispersion in Conception Bay, Newfoundland. Can. J. Fish. Aquat. Sci. **51**: 1297–1307.

Fahay, M.P. 1983. Guide to the early stages of marine fishes occurring in the western north Atlantic Ocean, Cape Hatteras to the southern Scotian Shelf. J. Northw. Atl. Fish. Sci. **4**: 1–423.

Fromentin, J.-M., Stenseth, N.C., Gjøsæter, J., Bjørnstad, O.N., Falck, W., and Johannessen, T. 1997. Spatial patterns of the temporal dynamics of three gadoid species along the Norwegian Skagerrak coast. Mar. Ecol. Prog. Ser. **155**: 209–222.

Goddard, S.V., Wroblewski, J.S., Taggart, C.T., Howse, K.A., Bailey, W.L., Kao, M.H., and Fletcher, G.L. 1994. Overwintering behaviour of adult northern cod (*Gadus morhua*) in cold inshore waters as evidenced by plasma antifreeze protein levels. Can. J. Fish. Aquat. Sci. **51**: 2834–2842.

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapp. P.-v. Réun. Cons. Int. Explor. Mer, **20**: 1–228.

Houde, E.D. 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. **2**: 17–29.

Hüssy, K., St. John, M.A., and Böttcher, U. 1997. Food resource utilization by juvenile Baltic cod *Gadus morhua*: a mechanism potentially influencing recruitment success at the demersal juvenile stage. Mar. Ecol. Prog. Ser. **155**: 199–208.

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Hutchings, J.A., and Myers, R.A. 1994. What can be learned from the collapse of a renewable resource? Atlantic cod, *Gadus morhua*, of Newfoundland and Labrador. Can. J. Fish. Aquat. Sci. **51**: 2126–2146.

Hutchings, J.A., Myers, R.A., and Lilly, G.R. 1993. Geographic variation in the spawning of Atlantic cod, *Gadus morhua*, in the northwest Atlantic. Can. J. Fish. Aquat. Sci. **50**: 2457–2467.

Kjesbu, O.S. 1994. Time of start of spawning in Atlantic cod *Gadus morhua* females in relation to vitellogenic oocyte diameter, temperature, fish length and condition. J. Fish Biol. **45**: 719–735.

Kulka, D.W., Wroblewski, J.S., and Narayanan, S. 1995. Recent changes in the winter distribution and movements of northern Atlantic cod (*Gadus morhua* Linnaeus, 1758) on the Newfoundland–Labrador Shelf. ICES J. Mar. Sci. **52**: 889–902.

Lear, W.H. 1984. Discrimination of the stock complex of Atlantic cod (*Gadus morhua*) off southern Labrador and eastern Newfoundland, as inferred from tagging studies. J. Northw. Atl. Fish. Sci. **5**: 143–159.

Lear, W.H., Fleming, A.M., and Wells, R. 1980. Results of small cod surveys in eastern Newfoundland during 1959–1964. North Atl. Fish. Org. SCR Doc. 80/1X/144.

May, A.W. 1967. Fecundity of Atlantic cod. J. Fish. Res. Board Can. **24**: 1531–1551.

Methven, D.A., and McGowan, C. 1998. Distinguishing small juvenile Atlantic cod (*Gadus morhua*) from Greenland cod (*G. ogac*) by comparison of meristic characters and discriminant function analyses of morphometric data. Can. J. Zool. **76**. In press.

Methven, D.A., and Schneider, D.C. 1998. Gear-independent patterns of variation in catch of juvenile Atlantic cod (*Gadus morhua*) in coastal habitats. Can. J. Fish. Aquat. Sci. **55**. 1430–1442.

Murphy, E. 1995. The status of 2GH cod, 3LNO haddock, 3Ps haddock and 3Ps pollock. DFO Atl. Res. Doc. 95/33.

Ouellet, P. 1997. Characteristics and vertical distribution of Atlantic cod (*Gadus morhua*) eggs in the northern Gulf of St. Lawrence, and the possible effect of cold water temperature on recruitment. Can. J. Fish. Aquat. Sci. **54**: 211–223.

Page, F.H., and Frank, K.T. 1989. Spawning time and egg stage duration in northwest Atlantic haddock (*Melanogrammus aeglefinus*) stocks with emphasis on Georges and Browns Bank. Can. J. Fish. Aquat. Sci. **46**: 68–81.

Pepin, P., Orr, D.C., and Anderson, J.T. 1997. Time to hatch and larval size in relation to temperature and egg size in Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 2–10.

Perry, R.I., and Neilson, J.D. 1988. Vertical distributions and trophic interactions of age-0 Atlantic cod and haddock in mixed and stratified waters of Georges Bank. Mar. Ecol. Prog. Ser. **49**: 199–214.

Peterman, R.M., Bradford, M.J., Lo, N.C.H., and Methot, R.D. 1988. Contribution of early life stages to interannual variability in recruitment of northern anchovy (*Engraulis mordax*). Can. J. Fish. Aquat. Sci. **45**: 8–16.

Pinsent, D.L., and Methven, D.A. 1997. Protracted spawning of Atlantic cod (*Gadus morhua*) in Newfoundland waters: evidence from otolith microstructure. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 19–24.

Rose, G.A. 1996. Cross-shelf distributions of cod in NAFO Divisions 2J3KL in May and June 1995: some preliminary findings of a longer term study. NAFO SCR Doc. 96/57.

Ruzzante, D.E., Taggart, C.T., Cook, D., and Goddard, S. 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua* L.) off Newfoundland: microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. **53**: 634–645.

Schneider, D.C., Methven, D.A., and Dalley, E.L. 1997. Geographic contraction in juvenile fish: a test with northern cod (*Gadus morhua*) at low abundances. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 187–199.

Shelton, P.A., Stansbury, D.E., Murphy, E.F., Lilly, G.R., and Brattey, J. 1996*a*. Assessment of the cod stock in NAFO Divisions 2J+3KL. NAFO SCR Doc. 96/62.

Shelton, P.A., Stansbury, D.E., Murphy, E.F., Lilly, G.R., and Brattey, J. 1996*b*. Assessment of the cod stock in NAFO Divisions 2J+3KL. DFO Atl. Fish. Res. Doc. 96/80.

Sissenwine, M.P. 1984. Why do fish populations vary? *In* Exploitation of marine communities. *Edited by* R.M. May. Springer-Verlag, Berlin. pp. 59–94.

Smedbol, R.K., and Wroblewski, J.S. 1997. Evidence for inshore spawning of northern Atlantic cod (*Gadus morhua*) in Trinity Bay, Newfoundland, 1991–1993. Can. J. Fish. Aquat. Sci. **54**(Suppl. 1): 177–186.

Taggart, C.T. 1997. Bank-scale migration patterns in northern cod. NAFO Sci. Counc. Stud. **29**: 51–60.

Taggart, C.T., Anderson, J., Bishop, C., Colbourne, E., Hutchings, J., Lilly, G., Morgan, J., Murphy, E., Myers, R., Rose, G., and Shelton, P. 1994. Overview of cod stocks, biology, and environment in the Northwest Atlantic region of Newfoundland, with emphasis on northern cod. ICES Mar. Sci. Symp. **198**: 140–157.

Taggart, C.T., Penny, P., Barrowman, N., and George, C. 1995. The 1954–1993 Newfoundland cod-tagging data base: statistical summaries and spatial–temporal distributions. Can. Tech. Rep. Fish. Aquat. Sci. No. 2042.

Trippel, E.A. 1998. Egg size and viability and seasonal offspring production of young Atlantic cod. Trans. Am. Fish. Soc. **127**: 339–359.

Wroblewski, J.S., Bailey, W., and Howse, K. 1994. Overwintering of adult Atlantic cod (*Gadus morhua*) in nearshore waters of Trinity Bay, Newfoundland. Can. J. Fish. Aquat. Sci. **51**: 142–150.

Wroblewski, J.S., Smedbol, R.K., Taggart, C.T., and Goddard, S.V. 1996. Behaviour of farmed and wild Atlantic cod (*Gadus morhua*) released in Trinity Bay, Newfoundland. Mar. Biol. **124**: 619–627.

Yao, T. 1986. The response of currents in Trinity Bay, Newfoundland, to local wind forcing. Atmos.-Ocean, **24**: 235–252.

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Stream phosphorus and nitrogen export from agricultural and forested watersheds on the Boreal Plain

Sandra E. Cooke and Ellie E. Prepas

Abstract: We evaluated phosphorus (P) and inorganic nitrogen (IN) export from two agricultural and two forested watersheds on the nutrient-rich but relief-poor Boreal Plain. One agricultural stream was in a watershed that contained cropland, while the second consisted of mixed agricultural activities. Over the 2-year study, total dissolved phosphorus (TDP) concentrations were proportionately high, particularly in the agricultural streams. Flow-weighted TDP averaged 82% of the total phosphorus (TP) in the agricultural streams and 43% in the forested streams. In all watersheds, TDP was almost exclusively dissolved reactive phosphorus and most of the annual P export was in summer. The type of agricultural activity in the watershed influenced IN speciation; in the mixed agricultural watershed, 94% of IN export was ammonium, whereas 98% of IN load was nitrate from the cropland watershed. Disproportionately high TDP to TP export from agricultural watersheds suggests that, in areas of low relief and relatively high soil water P content, land clearing may influence dissolved more than particulate phosphorus export.

Résumé : Nous avons évalué les exportations de phosphore (P) et d'azote inorganique (AI) en provenance de deux bassins hydrographiques à vocation agricole et de deux bassins hydrographiques boisés situés dans la plaine boréale, région riche en élément nutritifs mais peu accidentée. Dans les bassins hydrographiques à vocation agricole, un premier cours d'eau traversait des terres cultivés et un deuxième, des terres consacrées à des activités agricoles mixtes. Durant les deux années qu'a duré l'étude, les concentrations de phosphore total dissous (PTD) se sont révélées proportionnellement élevées, en particulier dans les cours d'eau situés en zone agricole. Les valeurs de PTD pondérées en fonction du débit s'élevaient en moyenne à 82 % du phosphore total (PT) dans les cours d'eau situés en régions agricoles, comparativement à 43 % dans les cours d'eau traversant des secteurs boisés. Dans tous les bassins hydrographiques, le PTD se trouvait presque exclusivement sous la forme de phosphore réactif dissous, et la majeure partie de l'exportation annuelle s'effectuait durant l'été. Le type d'activité agricole dans le bassin hydrographique influait sur la spéciation de l'AI : dans le bassin hydrographique à vocation agricole mixte, 94 % de l'AI se trouvait sous la forme d'ammonium, tandis que dans le bassin constitué de terres cultivées, 98 % de l'AI se présentait sous la forme de nitrate. La quantité disproportionnellement élevée de PTD exporté par rapport au PT provenant des bassins hydrographiques à vocation agricole permet de penser que, dans les secteurs peu accidentés où l'eau du sol renferme de fortes concentrations de P, le déboisement peut avoir un impact plus important sur l'exportation du phosphore dissous que sur celle du phosphore particulaire.

[Traduit par la Rédaction]

Introduction

Human activity in watersheds can increase phosphorus (P) and nitrogen (N) loading to surface waters and enhance primary production (Sharpley and Menzel 1987). Various studies have compared the impact of land-use on N and P export from watersheds, as influenced by watershed geology, physiography, hydrology, soil texture, and dominant vegetation (Dillon and Kirchner 1975; Omernik 1977; D'Arcy and Carignan 1997). Forested watersheds export less total P (TP) than watersheds with limited mixed agriculture and forest (Dillon and Kirchner 1975). In the United States, TP and total inorganic nitrogen (TIN) export from agricultural land can be up to 3 and 12 times higher, respectively, than from forested land (Omernik 1977). Although P and N export have been extensively documented for central and eastern regions of Canada and the United States, few export data exist for the low-relief watersheds located in glacial till in the Boreal Plain ecozone of western Canada. The latter is a region where high P content and relatively shallow depth of lakes makes them particularly vulnerable to small increases in biologically available phosphorus (Prepas and Trimbee 1988).

Phosphorus export from watersheds is routinely expressed as TP. Previous studies have established a strong empirical relationship between TP and chlorophyll *a* concentrations in lakes (e.g., Dillon and Rigler 1974; Prepas and Trew 1983). Generally, >50% of the TP in surface runoff from cultivated and forested land is particulate P (PP) (Omernik 1977;

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Sharpley and Menzel 1987; Vaithiyanathan and Correll 1992). However, most PP is unavailable for immediate algal uptake, or it acts as a long-term source of P to lakes (Sharpley and Menzel 1987). Thus, measurements of more biologically available P, such as total dissolved P (TDP) and dissolved reactive P (DRP), may increase the predictive power of lake production models if used as variables (Boström et al. 1988; Sharpley et al. 1991). Although the form of P introduced into a lake will influence the response of the primary producers, few studies have quantified TDP and DRP export from watersheds. If there are regions where clearing of forested land is likely to enhance dissolved phosphorus concentration in runoff, the Boreal Plain is likely to be one (D'Arcy and Carignan 1997). Relief in this region is low and soil water phosphorus concentration high (J. Evans, University of Alberta, Edmonton, unpublished data) in watersheds cleared for agriculture as compared with those still forested.

We undertook the present study to test the hypothesis that, in streams on the Boreal Plain, TDP would increase relative to PP. Our 2-year study focused on two nonintensive agricultural and two forested watersheds all located in a geologically similar region. Samples were taken flow proportionately (i.e., more frequently during peak flow periods than during low flow periods) to capture nutrient pulses during runoff events.

Materials and methods

Study site and hydrology

Four subwatersheds, two agricultural (A1 and A2) and two forested (F1 and F2), are located in northcentral Alberta in the Baptiste Lake watershed (54°45′N; 113°33′W) (Fig. 1). Watersheds F1, F2, and A1 drain into Baptiste Lake while A2 drains into the outflow, Baptiste Creek. The watershed areas are 57, 60, 3.8, and 6.6 km^2, respectively. The terrain is gently sloping (4–7%). Dominant soil types in this region are Dark Gray – Orthic Gray Luvisols of lacustrine origin, overlying glacial till of 30- to 100-m thickness (Kjearsgaard 1972). Approximately one third of the forested watersheds consists of poorly drained organic soils in depressional areas. The watersheds are located in the mixedwood boreal forest, a region that contains a mixture of relatively undisturbed forested areas and light agriculture. The predominant tree species in the uplands are trembling aspen (*Populus tremuloides*) and balsam poplar (*Populus balsamifera*), while in depressional areas the most common species are white and black spruce (*Picea mariana* and *Picea glauca*). Changes to vegetation in the forested watersheds were minimal during the study period.

Watersheds (A1 and A2) are typical of agricultural land use in the region. Nonrow cropland planted with barley (*Hordeum vulgare*) and canola (*Brassica napus* L.) is the predominant land use in watershed A1 (approximately 80% of the land area). The remainder of A1 consists of small woodlots and one homestead. Land-use in watershed A2 consists of mixed agriculture with nonrow cropland (34%) and two cow–calf operations of approximately 100 and 25 head (25%). The remainder of A2 consists of small woodlots and two homesteads. Cropland fertilizer application rates within A1 and A2 averaged 48 kg $N \cdot ha^{-1}$ and 23 kg $P \cdot ha^{-1}$, respectively for 1994–1995 (Alberta Agriculture, 7000–113 Street, Edmonton, AB T6G 5T6, Canada). In watershed A2, cattle were wintered and fed next to the stream and had uncontrolled access to the stream channel.

The streams in the forested watersheds are second order and supported many (>10) beaver dams, whereas the agricultural watersheds contained first-order, intermittent streams that were void of any beaver activity. Small intermittent streams that experience streamflow only after runoff events (e.g., snowmelt or rainfall events) are common in agricultural areas on the Boreal Plain, where the lack of permanent vegetation may contribute to the lack of permanent stream flow.

For this study, we searched for streams (*i*) in the same geological setting, some being cleared for agriculture and others, still forested; (*ii*) located on the Boreal Plain; (*iii*) accessible by road; and (*iv*) within 1 h drive of the Meanook Biological Field Station, the latter to allow for almost instantaneous processing of water samples for time-dependent analyses. Although these streams reflect the inevitable differences found between experiments involving natural systems, they were the best experimental sites we could find in this region of the Boreal Plain. The watershed areas of the agricultural streams were, on average, just less than 10% of the forested streams. We know of no evidence to suggest that small streams export necessarily more or less phosphorus than larger ones, per unit area, at this scale. Cross (1977) studied 16 streams in a forested drainage basin on the Precambrian Shield. Watersheds of the five largest streams varied in area from 1 to 19 km^2. In her study, export from these larger streams varied little (9.7–13.9 $kg \cdot km^{-2} \cdot year^{-1}$).

In our study, all sampling stations were located upstream of the stream–lake confluence to avoid backflow from the lake. There were 2 cow-calf operations on stream A2; on this stream an additional sampling site was located upstream of the 100-head cow–calf operation (A2 upstream) but downstream of the second and smaller 25-head cow–calf operation. Streams were sampled from May to October (summer runoff) in 1994 and from March to September (spring and summer runoff) in 1995. Stream discharge was quantified in F1, F2, and A2 by measuring current velocity and water depth at set intervals across each stream channel with a Gurley pygmy current meter. In 1994, stream surface velocity in A1 was measured by observing the time required for a float to traverse a known distance downstream as the stream channel was poorly defined. Stream discharge estimates were corrected by a factor of 0.80 for rough stream beds after Gordon et al. (1994). In 1995, a 90° V-notch weir was installed in A1 to estimate stream discharge. A stream discharge rating curve for A1 was established based on

$$(1) \qquad Q = 1.34H^{2.48}$$

where Q is discharge ($m^3 \cdot s^{-1}$) and H is upstream depth or head (m) (Gordon et al. 1994), verified through timed volumetric sampling of water flowing over the weir. Stream discharge at the A2 upstream site was measured volumetrically as runoff was channelled to a natural overfall. Stream stage was recorded during each site visit in 1994, while automatic stage recorders (Reedyk et al. 1997), were employed during 1995. Stage measurements were compared with measured discharge over a large range of stream flows to construct stage–discharge relationships for each stream.

Water samples were collected at set intervals for the agricultural and forested watersheds with an ISCO automatic sampler during periods of high flow. Since stream discharge peaks were steeper and of shorter duration in the agricultural streams, autosamplers were programmed to collect water samples at 2-h intervals during runoff events. Automatic samplers were programmed to collect water samples at 4-h intervals during runoff events in the forested watershed. During spring runoff, autosamplers were programmed to sample at 2- and 4-h intervals between 08:00 and 18:00 as the streams usually froze at night. Additional grab samples were collected approximately every 2 days during low flow periods and three or four times daily during storm events. Grab samples were collected in the middle of each stream at mid-depth with acid-

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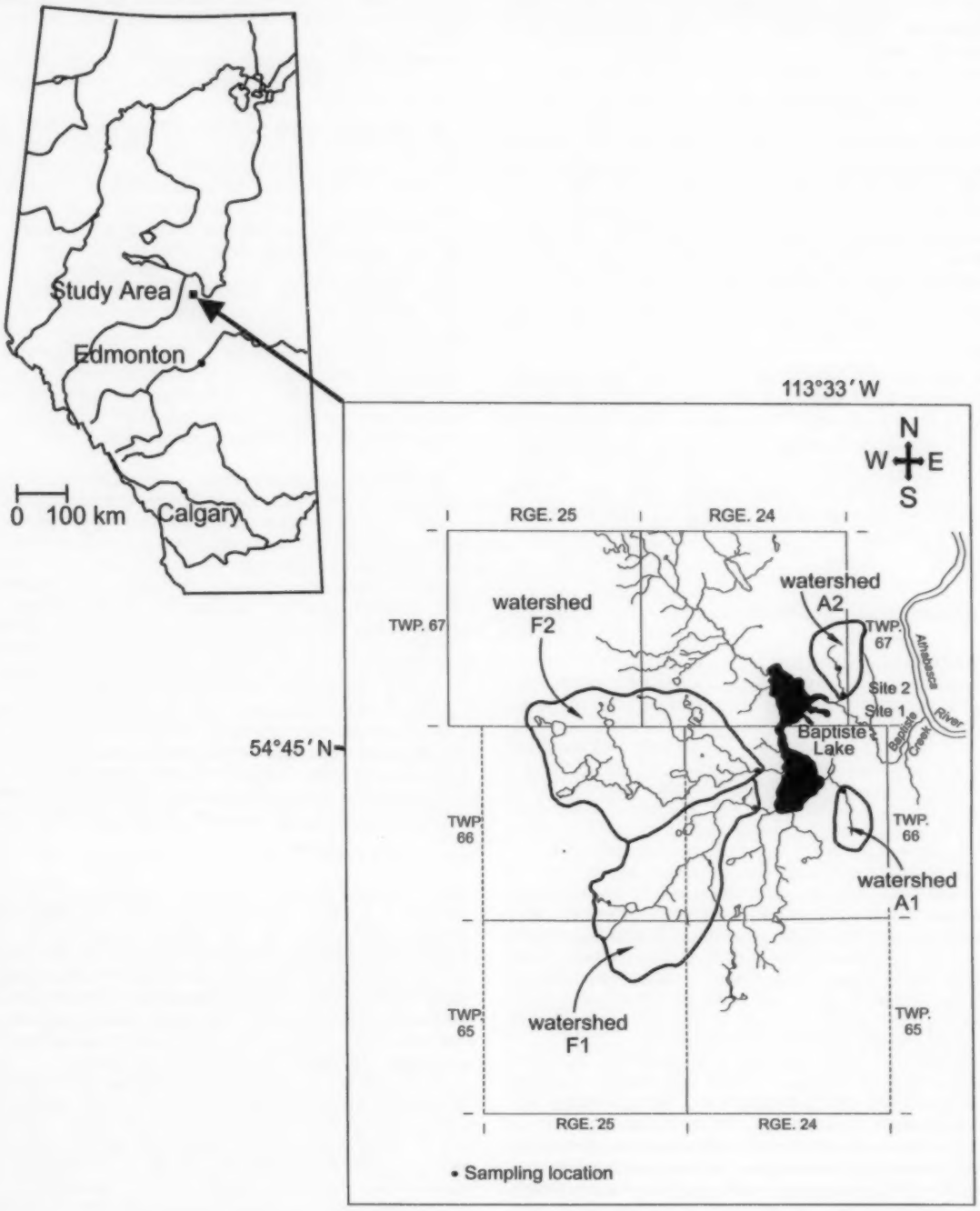
Fig. 1. Map of the Baptiste Lake watershed in north-central Alberta, Canada, showing location of the two forested (F1 and F2) and two agricultural (A1 and A2) watersheds.



washed polystyrene and Nalgene polyethylene containers. Water samples were stored on ice in the field and refrigerated at 4°C in the laboratory prior to analysis.

Water chemistry and nutrient loads

Water samples taken by the automatic samplers were analyzed discretely during peak flows and pooled and analyzed as daily composites for low-flow periods. Water samples collected in the automatic samplers were analyzed for TP and TDP only, as NO_3^-, NH_4^+, and DRP are less stable and require immediate analysis. Therefore, NO_3^-, NH_4^+ and DRP were measured exclusively by grab samples. TP and TDP were measured on all water samples

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Table 1. Nutrient export coefficients ($kg \cdot km^{-2} \cdot year^{-1}$) for the agricultural (A) and forested (F) watersheds.

	1994			1995					
Watershed	TP	TDP	PP	TP	TDP	PP	DRP	NO_3^-	NH_4^+
F1	13	6.5	6.5	9	3	6	2	3	1
F2	22	11	11	5	2	3	2	1	1
A1	14	11	3	12	8	4	7	29	2
A2	82	75	7	57	51	6	51	4	158
A2 (upstream)	57	49	6	34	30	4	18	8	40

Note: DRP, NO_3^-, and NH_4^+ were sampled less frequently than TP and TDP and are for 1995 only.

collected during 1994 and 1995, while water samples collected in 1995 were analyzed for NO_3^-, NH_4^+ and DRP in addition to TP and TDP. Consequently, NO_3^-, NH_4^+, and DRP were measured less frequently than TP and TDP. All grab samples were prepared and analyzed within 24 h of collection; samples collected by automatic samplers were analyzed within 72 h.

Total P and NH_4^+ concentrations were determined from unfiltered stream water, whereas TDP, DRP, and NO_3^- concentrations were determined on water samples filtered through a prerinsed 0.45-μm Millipore HAWP membrane filter. Total P and TDP were analyzed according to Menzel and Corwin's (1965) potassium persulfate method as modified by Prepas and Rigler (1982); DRP was determined according to Murphy and Riley's (1962) molybdenum blue method on undigested samples. Particulate P concentration was calculated as the difference between TP and TDP concentration. NH_4^+ and NO_3^- were analyzed on an autoanalyzer according to Solórzano's (1969) phenolhypochlorite method and the Stainton et al. (1977) cadmium–copper reduction method, respectively. Total inorganic N concentrations were calculated as the sum of NH_4^+ and NO_3^- concentrations. Stream pH and conductivity were measured in the laboratory.

Total nutrient load was calculated as the sum of the product of nutrient concentration and estimated discharge volume over each sampling interval (Munn and Prepas 1986; Ng et al. 1993). Nutrient export coefficients were computed by dividing the total nutrient load (kg) by watershed area (km^2). Monthly and annual nutrient flow-weighted mean concentrations (FWMCs) were calculated for each stream. FWMCs are calculated by dividing the mass load estimate by the total flow volume for a given period of time.

During spring runoff (March to April) in 1994, P loads for all watersheds were calculated based on estimated stream discharge and total volumes. A rating equation was used to extrapolate Water Survey of Canada (WSC) stream discharge data in watershed F1 to the sampling sites in watersheds F1 and F2. Similarly, a relationship between area yield of runoff was established between the WSC gauging station in F1 and the agricultural watersheds with 1994 and 1995 stream discharge data. Estimated stream discharges for F1 and F2 and runoff volumes for A1 and A2 were used to calculate TP, TDP, and PP loads during spring runoff in 1994. Estimated mass P loads were added to the mass P loads calculated from stream discharge and P data for each watershed to produce total annual mass P loads for 1994.

Statistical analysis

All data were log transformed prior to statistical analysis to satisfy normality and homogeneity of variance criteria. Monthly FWMCs of TP, TDP, and PP for each watershed were compared using *t* tests to detect differences between sampling years. Differences in monthly FWMCs between watersheds during 1994 and 1995 were determined by analysis of variance (ANOVA); Bonferroni multiple comparison tests identified differences between watersheds for P FWMCs. The least significant difference multiple comparison test determined pairwise differences, unadjusted for error rate, between monthly FWMCs of NO_3^- and NH_4^+. Statistical analyses were performed on SPSS (version 6.0 for Windows) software (Norusis 1993).

Results

Hydrology

Total annual precipitation in the study area during 1994 and 1995 was 562 and 548 mm respectively and was above the long-term (1960–1995) average (496 mm). Generally, depth of runoff was greater during 1994 than 1995, with greater areal runoff from forested than agricultural watersheds. The depth of runoff for the forested watersheds (F1 and F2, respectively) was 103 and 128 mm in 1994 and 25 and 46 mm in 1995; for the agricultural watersheds (A1 and A2, respectively) it was 49 to 67 mm in 1994 and 31 and 39 in 1995. Historically, there is a considerable amount of variability between precipitation and runoff in the forested watershed F1. The large variability in the depth of runoff between years in the forested watersheds may be due to beaver activity upstream of the gauging stations.

Nutrient loads

Most of the phosphorus export from the agricultural watersheds was in the dissolved form (Table 1). TDP export from cropland was almost twice that of the mean of the two forested watersheds, while TDP export from the mixed agricultural watershed was almost nine times greater than that of the forested watersheds. The mixed agricultural watershed exported five times more TDP than the watershed predominated by cropland. Conversely, PP export was similar among agricultural and forested watersheds. In addition, TDP export exceeded PP export from the agricultural watersheds, whereas PP export was similar to TDP export from the forested watersheds. Overall, the agricultural watersheds exported the greatest mass of TDP.

Our 1995 data indicate that most TDP exported from the agricultural and forested watersheds was DRP. However, the agricultural watersheds exported 3–25 times more DRP than the forested watersheds. Of the two agricultural watersheds sampled, the mixed agricultural watershed (A2) exported the greatest mass of DRP.

Runoff from summer storm events delivered greater nutrient loads from cropland and forested watersheds than spring runoff (Fig. 2). Summer runoff comprised approximately 61% of total runoff volume from forest land and supplied an average of 50 and 74% of the total NH_4^+ and TP load, respectively, whereas 33% of the total NO_3^- load was delivered in baseflow. Similarly, summer runoff from cropland supplied 93% of the total runoff volume and contributed 65, 91, and 96% of the total NO_3^-, TP, and NH_4^+ load, respec-

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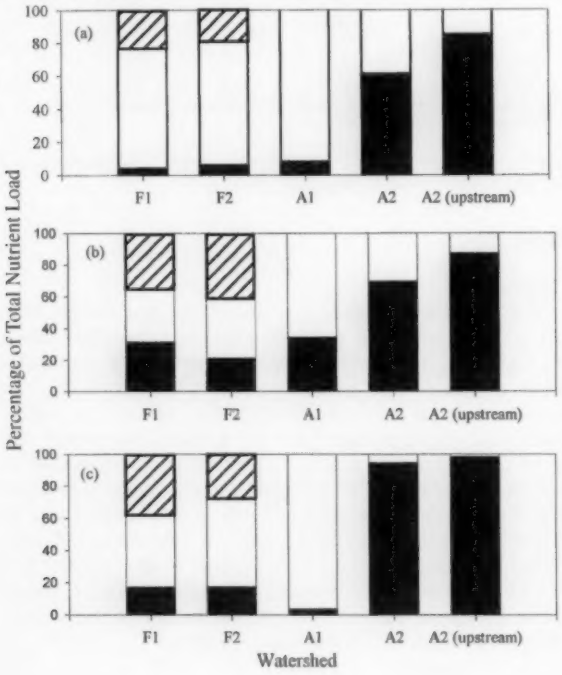
Fig. 2. For streams draining the forested (F1 and F2) and agricultural (A1 and A2) watersheds, the percentage of the total nutrient load transported in spring runoff (solid bars), summer runoff (open bars), and baseflow (hatched bars) during 1995 for (*a*) total phosphrous, (*b*) nitrate, and (*c*) ammonium.



tively. In contrast, most of the nutrient load from the mixed agricultural watershed was delivered in spring runoff; it supplied 75, 83, and 97% of the total TP, NO_3^-, and NH_4^+ load, respectively, in less than 50% of the total runoff volume. Although most of the runoff, and consequent nutrient export, occurred during the summer from cropland and forest land, spring runoff contributed more nutrients in a lower volume of runoff from the mixed agricultural watershed with two cow–calf operations. This spring pulse was likely the result of melting and subsequent flushing of animal wastes that had accumulated over the winter.

Annual TDP FWMCs were eight times higher in the agricultural streams relative to the mean of the forested streams, whereas annual PP FWMCs were similar between the agricultural and forested streams (Table 2). Annual NO_3^- FWMCs in the stream draining cropland and annual NH_4^+ FWMCs in the stream draining mixed agriculture were approximately 10 and 20 times greater, respectively, than the mean of the corresponding FWMCs in streams draining the forested watersheds.

Monthly TDP and PP FWMCs for each watershed were not detectably different between years ($0.08 < P < 0.90$, data not shown). Stream water draining cropland and the mixed agricultural watershed contained higher TDP concentrations relative to the forested watersheds ($P < 0.001$). TDP FWMCs from the mixed agricultural watershed, downstream of the 100-head cow–calf operation, were greater than either the cropland or forested watersheds. Monthly TDP FWMCs measured in the mixed agricultural watershed downstream of the 25-head cow–calf operation (A2 upstream) were similar to monthly FWMCs measured in cropland runoff. TDP concentrations were higher in F2 than F1 ($P < 0.05$). In contrast, stream PP monthly FWMCs were not detectably different among the four watersheds ($P = 0.08$). In general, monthly FWMCs of P in the dissolved fractions were higher in stream water draining agricultural than forested watersheds.

Agricultural practices in the watershed appeared to influence the fractionation of P in stream water. Most of the P in streams draining the agricultural watersheds was in the dissolved form. TDP comprised, on average, 75 and 90% of the TP pool in runoff from the cropland and mixed agricultural watersheds, respectively (Fig. 3). In contrast, TDP made up less than half (36–47%) of the TP pool in streams draining forest land. Much (76–100%) of the TDP in the streams draining the forested and agricultural watersheds was DRP.

The agricultural watersheds exported up to 50 times more TIN than the forested watersheds (Fig 4). Agricultural practices also influenced the fractionation of IN in runoff from agricultural watersheds. NO_3^- was the predominant form of IN in runoff draining cropland, comprising 98% of the TIN pool. In contrast, NH_4^+ was the main form of IN exported from the mixed agricultural watershed, making up 94% of the TIN pool. Conversely, NO_3^- comprised approximately half of the TIN pool in stream water draining forest land.

TIN concentrations were highly variable in stream water draining the cropland and mixed agricultural watersheds (data not shown). During spring runoff, instantaneous NO_3^- concentrations approached 10 $mg \cdot L^{-1}$ in runoff from cropland and instantaneous NH_4^+ concentrations exceeded 27 $mg \cdot L^{-1}$ in the stream draining mixed agriculture. Monthly NO_3^- FWMCs in stream water draining cropland were high relative to NO_3^- concentrations in runoff from the mixed agricultural watershed ($P = 0.002$). Because of the high month-to-month variability in NH_4^+ FWMCs, no differences were detected between the agricultural watersheds. Monthly FWMCs of NO_3^- and NH_4^+ were also not detectably different ($P > 0.05$) in streams draining the forested watersheds.

Discussion

Most (67–91%, 60–91%, respectively; Table 1) of the P export was in the dissolved (i.e., TDP and DRP) fraction from our two agricultural watersheds. The predominance of TDP in runoff from watersheds on the Boreal Plain may be due to watershed characteristics such as low-sloping topography, which results in longer leaching times and a high proportion of organic soils. The high proportion of TDP in runoff from agricultural watersheds on the Boreal Plain suggests that P–soil–water interactions may differ from other regions of North America where PP export dominates.

High TDP in runoff from agricultural watersheds, specifically cropland, on the Boreal Plain may be due to unique geochemical processes, the erosion potential of regional soils, and the pH of runoff. The pH of stream water draining the agricultural and forested watersheds in this study ranged from 7.0 to 7.6. Since HPO_4^{2-} and $H_2PO_4^-$ ions predominate in runoff with circumneutral pH (Ng et al. 1993), P would tend to remain in the dissolved fraction as the adsorp-

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Table 2. Annual FWMCs ($\mu g \cdot L^{-1}$) for the agricultural (A) and forested (F) watersheds (1994 FWMCs do not include spring runoff).

Watershed	Year	TP	TDP	PP	DRP	NO_3^-	NH_4^+
F1	1994	123	62	61	—	—	—
	1995	187	50	137	38	65	30
F2	1994	170	81	89	—	—	—
	1995	198	93	105	74	34	39
A1	1994	434	351	83	—	—	—
	1995	396	275	121	229	968	49
A2	1994	702	637	65	—	—	—
	1995	1119	994	125	993	151	2359
*A2 (upstream)	1994	464	389	45	—	—	—
	1995	839	737	102	436	196	979

Note: DRP, NO_3^-, and NH_4^+ were sampled less frequently than TP and TDP and are for 1995 only.

Fig. 3. Percentage of the total phosphorus (TP) pool comprised of total dissolved phosphorous (TDP) (solid bars) and particulate phosphorus (PP) (open bars) in the streams draining the forested (F1 and F2) and agricultural (A1 and A2) watersheds.

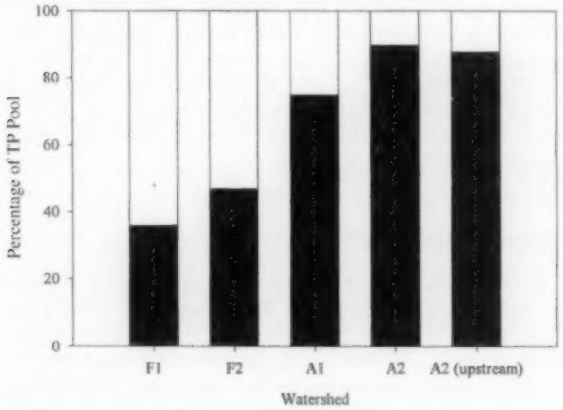
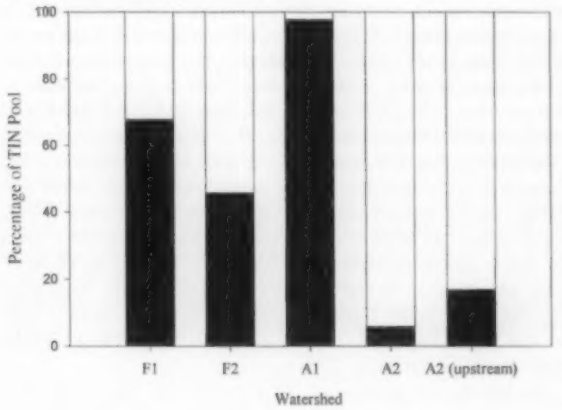


Fig. 4. Percentage of the total inorganic nitrogen (IN) pool comprised of nitrate (NO_3^-) (solid bars) and ammonium (NH_4^+) (open bars) in streams draining the forested (F1 and F2) and agricultural (A1 and A2) watersheds.



tion of P onto suspended sediment for transportation as PP would be limited. Furthermore, TDP generally dominates in runoff from forest or grassland with nonerosive soils that carry little sediment (Sharpley et al. 1994). Although suspended sediment was not measured in this study, the predominance of TDP in runoff from agricultural watersheds on the Boreal Plain may also be due, in part, to a low suspended sediment load and subsequently low PP load in runoff from the non-intensive agriculture in this region. Other regions with more intensive agriculture (i.e., row crops) have higher suspended sediment loads contributing to an overall greater P export with a greater proportion as PP than TDP (Sharpley et al. 1981). Because TDP predominates in runoff from agricultural lands on the Boreal Plain, typical erosion control measures such as grassed filter strips may not be sufficient to reduce dissolved P inputs to aquatic systems.

Measurements of dissolved P in runoff from agricultural watersheds may more accurately represent the potential impacts of agriculture on bioavailable phosphorus and thus aquatic primary productivity than TP. Although PP can be a long-term source of P for aquatic biota (Carignan and Kalff 1980), TDP is often more readily available for biological uptake (Nurnberg and Peters 1984). Although DRP does not represent all biologically available P in freshwater (Peters 1977), at high concentrations (i.e., >30 $\mu g \cdot L^{-1}$), TDP and DRP are thought to be immediately available for biological uptake (Bradford and Peters 1987; Boström et al. 1988). Since TDP and DRP concentrations in runoff from the agricultural watersheds were very high, most of the P may be immediately available for algal uptake. This notion is supported by data from two Alberta lake studies: (*i*) when DRP concentrations are high (>8 $mg \cdot L^{-1}$), orthophosphate turnover times indicate that the DRP is biologically available (Prepas 1983), and (*ii*) phosphorus in runoff during a one in 100-year storm in the same region as the Baptiste Lake study was almost entirely biologically available (Prepas and Trimbee 1988). Therefore, high levels of P in runoff from agricultural watersheds will almost certainly accelerate the eutrophication of surface waters on the Boreal Plain.

Seasonal P export patterns for the forested and cropland agricultural streams in this study are consistent with a previous methodologically comparable study (Munn and Prepas 1986) carried out a decade earlier on two more forested streams also on the Boreal Plain. Most (74–95%) of the annual P export from five of the watersheds from both studies

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was in summer. High summer P export in these streams was associated with summer storms and the traditional high summer runoff (61–93% of total runoff). These watersheds vary in size from first to fourth order (3.8–281 km^2). Thus, the seasonal patterns observed are consistent across local streams for at least these four orders. Further, high P export is during a period when phytoplankton communities are susceptible to algal blooms including cyanobacterial toxins (Prepas and Trew 1983). Thus, increases in P export during this critical summer period are likely to have biological and water quality implications.

Although P concentrations were higher in the agricultural watersheds than in the forested watersheds, P concentrations and the relative proportion of TDP in streams draining the forested watersheds were high relative to those draining the Precambrian Shield (Dillon and Kirchner 1975) and a previous study on the Boreal Plain. The characteristics of watersheds on the Boreal Plain such as vegetation and soil type likely contribute to overall high P loading. Leaching from Gray Luvisolic soils is an important mechanism for P flux and removal in northern boreal forest ecosystems and is much greater than P loss from other soils (Frossard et al. 1989; Xiao et al. 1991). Interestingly, the proportion of TDP in the two forested streams in this study was higher than in the previous stream study in the Boreal Plain (Munn and Prepas 1986; mean 43 vs. 25%). The obvious difference between these two sets of streams is watershed vegetation; the current study watersheds were aspen dominated, while the former study watersheds were coniferous dominated. Thus, forest cover, as well as other watershed characteristics such as slope, likely influence the relatively high proportion of TDP observed in stream water in our study. The high proportion of PP in our forested versus agricultural streams may be due to the high leaf litter in aspen forested versus cleared watersheds (Xiao et al. 1991).

Within-stream processing of nutrients by plants and microbial life in streams can also influence nutrient export. However, inputs entering during high discharges (i.e., spring and summer runoff) may be flushed out with minimal entry to biological or geochemical pathways (Meyer and Likens 1979). The forested watersheds in this study contained longer, permanent streams with a greater potential for within-stream processing than the agricultural streams. However, comparisons of nutrient loads between the agricultural and forested streams were primarily based on samples collected during high discharges, when within-stream processing is at its lowest (also Munn and Prepas 1986).

Total IN export from agricultural watersheds exceeded export from forested watersheds by an order of magnitude. Furthermore, the speciation of N differed according to agricultural land use: NO_3^- was the dominant N species in runoff from cropland, whereas NH_4^+ predominated in runoff from the mixed agricultural watershed with two cow–calf operations. NO_3^- is very mobile and can easily leach through the soil profile (Marston 1989). Bacterial nitrification of excess N fertilizer (i.e., anhydrous ammonia) to NO_3^-, likely contributed to the high NO_3^- export from cropland (e.g., Hill 1978; Lucey and Goolsby 1993). Conversely, high NH_4^+ export during spring runoff from the mixed agricultural watershed with two cow–calf operations suggests that much of the organic nitrogen from livestock waste that accumulated over the winter was converted to ammonia (ammonification) in runoff. This is likely due to cold temperatures over winter and during spring runoff that do not facilitate further bacterial breakdown of NH_4^+ to NO_3^-. Therefore, excessive N fertilizer or poor management of manure can contribute to high N export from a watershed. Since certain cyanobacteria are N limited, high N export (NH_4^+ and NO_3^-) from agricultural watersheds could increase primary productivity and accelerate the eutrophication of surface waters on the Boreal Plain.

Agriculture on the Boreal Plain affects surface water quality by increasing P and N loads. Because of the high proportion of dissolved P transported in runoff from agricultural land, erosion-control measures designed to reduce PP export would be inadequate to reduce dissolved P loads to downstream water bodies on the Boreal Plain. Further research is required to determine why P is primarily exported in the dissolved fraction from agricultural watersheds in this region. Also, it is evident that cattle with direct access to streams, a common practice in this region, can substantially increase nutrient loads in streams and, therefore, may have substantive effects on downstream water quality. Although P concentrations are high in forested streams on the Boreal Plain, agricultural activities increase P and N levels. Research is required to define the impacts of various land uses, such as forest harvesting, as the Boreal Plain is under increasing pressure from human activities.

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References

Boström, B., Persson G., and Broberg B. 1988. Bioavailability of different phosphorus forms in freshwater. Hydrobiologia, **170**: 133–155

Bradford, M.E., and Peters, R.H. 1987. The relationship between chemically analyzed phosphorus fractions and bioavailable phosphorus. Limnol. Oceanogr. **32**: 1124–1137.

Carignan, R., and Kalff, J. 1980. Phosphorus sources for aquatic weeds: water or sediment? Science (Washington, D.C.), **207**: 987–989.

Cross, P.M. 1977. An attempt to estimate phosphorus retention in Bob Lake and the iron:phosphorus ratio in the sediments. M.Sc. thesis, University of Toronto, Toronto, Ont.

D'Arcy, P., and Carignan, R. 1997. Influence of catchment topography on water chemistry in southeastern Quebec Shield lakes. Can. J. Fish. Aquat. Sci. **54**: 2215–2227.

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Dillon, P.J., and Kirchner, W.B. 1975. The effects of geology and land use on the export of phosphorus from watersheds. Water Res. **9**: 135–148.

Dillon, P.J., and Rigler, F.H. 1974. The phosphorus–chlorophyll relationship in lakes. Limnol. Oceanogr. **19**: 767–773.

Frossard, E., Stewart, J.W.B., and St Arnaud, R.J. 1989. Distribution and mobility of phosphorus in grassland and forest soils of Saskatchewan. Can. J. Soil Sci. **69**: 401–416.

Gordon, N.D., MacMahon, T.A., and Finlayson, B.L. 1994. Stream hydrology, an introduction for ecologists. John Wiley & Sons, Toronto, Ont.

Hill, A.R. 1978. Factors affecting the export of nitrate-nitrogen from drainage basins in southern Ontario. Water Res. **12**: 1045–1057.

Kjearsgaard, A.A. 1972. Soil survey of the Tawatinaw map sheet (83-I). Alta. Inst. Pedol. Rep. No. S-72-29. University of Alberta Press, Edmonton.

Lucey, K.J., and Goolsby, D.A. 1993. Effects of climatic variations over 11 years on nitrate-nitrogen concentrations in the Raccoon River, Iowa. J. Environ. Qual. **22**: 38–46.

Marston, R.A. 1989. Particulate and dissolved losses of nitrogen and phosphorus from forest and agricultural soils. Prog. Phys. Geogr. **13**: 234–259.

Menzel, D.W., and Corwin, N. 1965. The measurement of total phosphorus in seawater based on the liberation of organically bound fractions by persulfate oxidation. Limnol. Oceanogr. **10**: 280–282.

Meyer, J.L., and Likens, G.E. 1979. Transport and transformations of phosphorus in a forest stream ecosystem. Ecology, **60**: 1255–1269.

Munn, N., and Prepas, E.E. 1986. Seasonal dynamics of phosphorus partitioning and export in two streams in Alberta. Can. J. Fish. Aquat. Sci. **43**: 2464–2471.

Murphy, J.H., and Riley, J.P. 1962. A modified single solution method for the determination of phosphorus in natural waters. Anal. Chim. Acta, **27**: 31–36.

Ng, H.Y.F., Mayer, T., and Marsalek, J. 1993. Phosphorus transport in runoff from a small agricultural watershed. Water Sci. Technol. **28**: 451–460.

Norusis, M.J. 1993. SPSS for Windows, base system user's guide, release 6.0. SPSS Inc., Chicago, Ill.

Nurnberg, G.K., and Peter,. R.H. 1984. Biological availability of soluble reactive phosphorus in anoxic and oxic freshwaters. Can. J. Fish. Aquat. Sci. **41**: 757–765.

Omernik, J.M. 1977. Nonpoint source – stream nutrient level relationships: a nationwide study. PA-600/3-77-105. U.S. Environmental Protection Agency, Corvallis, Oreg.

Peters, R.H. 1977. Concentrations and kinetics of phosphorus fractions in water from streams entering Lake Memphremagog. J. Fish. Res. Board Can. **35**: 315–328.

Prepas, E.E. 1983. Orthophosphate turnover time in shallow productive lakes. Can. J. Fish. Aquat. Sci. **40**: 1412–1418.

Prepas, E.E., and Rigler, F.H. 1982. Improvements in quantifying the phosphorus concentration in lake water. Can. J. Fish. Aquat. Sci. **39**: 822–829.

Prepas, E.E., and Trew, D.O. 1983. Evaluation of the phosphorous–chlorophyll relationship for lakes of the Precambrian Shield in western Canada. Can. J. Fish. Aquat. Sci. **40**: 27–35.

Prepas, E.E., and Trimbee, A.M. 1988. Evaluation of indicators of nitrogen limitation in deep prairie lakes with laboratory bioassays and limnocorrals. Hydrobiologia, **159**: 269–276.

Reedyk, S., Cooke, S.E., Burchett, G., and Prepas, E.E. 1997. Design for an inexpensive continuous digital output water level recorder. Water Resour. Res. **33**: 1523–1526.

Sharpley, A.N., and Menzel, R.G. 1987. The impact of soil and fertilizer phosphorus on the environment. Adv. Agron. No. 41. pp. 297–324.

Sharpley, A.N., Menzel, R.G., Smith, S.J., Rhoades, E.D., and Olness, A.E. 1981. The sorption of soluble phosphorus by soil material during transport in runoff from cropped and grassed watersheds. J. Environ. Qual. **10**: 211–215.

Sharpley, A.N., Troeger, W.W., and Smith, S.J. 1991. The measurement of bioavailable phosphorus in agricultural runoff. J. Environ. Qual. **20**: 235–238.

Sharpley, A.N., Chapra, S.C., Wedepohl, R., Sims, J.T., Daniel, T.C., and Reddy, K.R. 1994. Managing agricultural phosphorus for protection of surface waters: issues and options. J. Environ. Qual. **23**: 437–451.

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. **14**: 799–801.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of freshwater. 2nd. ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. No. 25.

Vaithiyanathan, P., and Correll, D.L. 1992. The Rhode River watershed: phosphorus distribution and export in forest and agricultural soils. J. Environ. Qual. **21**: 280–288.

Xiao, X.J., Anderson, D.W., and Bettany, J.R. 1991. The effect of pedogenetic processes on the distribution of phosphorus, calcium and magnesium in Gray Luvisols. Can. J. Soil Sci. **71**: 397–410.

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Expression of viral hemorrhagic septicemia virus in prespawning Pacific herring (*Clupea pallasi*) exposed to weathered crude oil

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Abstract: Expression of subclinical viral infection in response to toxicant exposure has not previously been reported, but evidence presented herein indicates that activation of viral hemorrhagic septicemia virus (VHSV) may occur in Pacific herring (*Clupea pallasi*) exposed to crude oil encountered after an oil spill. Decreased incidence of hepatic inflammatory cells as a function of total polynuclear aromatic hydrocarbon (TPAH) concentration was evidence for immunosuppression in exposed fish, and decreased immune surveillance is a possible mechanism by which subclinical VHSV could be reactivated. Adult Pacific herring of unknown status regarding infection by VHSV were captured from the wild and exposed to weathered crude oil for 16–18 days. TPAH concentration in tissue, VHSV prevalence, and mortality were correlated with dose. Histopathologic lesions were significantly correlated with TPAH concentration and prevalence of VHSV, but not gender or length. Significant lesions included increased hepatocellular necrosis, splenic thrombosis, and decreased inflammation in the liver.

Résumé : Les résultats présentés dans cet article indiquent qu'une exposition au pétrole brut survenant à la suite d'un déversement peut activer le virus responsable de la septicémie hémorragique virale (VSHV) chez le hareng du Pacifique (*Clupea pallasi*). Jamais auparavant l'expression d'une infection virale infraclinique causée par l'exposition à des substances toxiques n'avait été signalée dans la littérature. La réduction du nombre de cellules inflammatoires hépatiques en fonction de la concentration des hydrocarbures aromatiques polycycliques totaux (HAPT) dénotait la présence d'une immunosuppression chez les poissons exposés. La diminution de la surveillance immunitaire pourrait être un mécanisme favorisant la réactivation du virus et l'apparition d'une infection infraclinique à VSHV. Des harengs du Pacifique dont on ignorait le statut à l'égard de l'infection à VSHV ont été capturés en mer et exposés pendant 16 à 18 jours à du pétrole brut altéré. Une corrélation a été établie entre la concentration de HAPT dans les tissus, la prévalence du VSHV et la mortalité, d'une part, et la dose, d'autre part. Une corrélation significative a également été observée entre la présence de lésions histopathologiques, d'une part, et la concentration de HAPT et la prévalence du VSHV, d'autre part. Aucune corrélation n'a cependant été notée entre la présence de lésions histopathologiques et le sexe et la longueur des poissons. Les lésions importantes observées incluaient les pathologies suivantes : augmentation de la nécrose hépatocellulaire, thrombose splénique et réduction de l'inflammation dans le foie.

[Traduit par la Rédaction]

Introduction

Establishment of causative links in wild fish among infectious disease, immune suppression, and environmental stress is difficult (Sindermann 1993). Many fish are asymptomatic carriers of pathogens that under normal conditions are held in check by the immune system. When that system is impaired by stress, the disease-causing agent may multiply and injure or kill its host (Anderson 1990). We hypothesize that oil spills may be one such stressor.

Oil spills occur frequently in marine systems throughout the world, and histopathology of the effects of hydrocarbon exposure on fish has been documented in laboratory and field studies (Haensly et al. 1982; Malins 1982; Solangi and Overstreet 1982). For example, prevalence and intensity of infections with gastrointestinal parasites were lower in fish treated with crude oil in the laboratory than in controls (Khan and Kiceniuk 1983), but other fish had increased numbers of branchial *Trichodina* after chronic exposure to oiled sediments (Khan 1990). Fourteen days after the *Exxon Valdez* oil spill, numbers of larval *Anisakis simplex* in the peritoneal cavity were lower in Pacific herring (*Clupea pallasi*) from two oiled sites than three unoiled sites; these results were confirmed in Pacific herring exposed to crude oil in the laboratory (Moles et al. 1993). Changes in expression of bacterial and viral pathogens after oil exposure have not been documented.

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Pacific herring sampled immediately after the *Exxon Valdez* oil spill from oiled sites had hepatic necrosis that was attributed to oil exposure (Moles et al. 1993). However, Pacific herring sampled annually from the same sites from 1990 through 1992 did not have hepatic necrosis and there was no longer any evidence of hydrocarbon exposure (Kocan et al. 1996). In PWS, in 1993, an abundant 1988 year-class was fully recruiting into the spawning population of Pacific herring, but the entire population collapsed. Of the remaining Pacific herring, 15–43% exhibited external ulceration or hemorrhages associated with viral hemorrhagic septicemia virus (VHSV) (Meyers et al. 1994), and fish again had hepatic necrosis. In 1994, VHSV isolated from individual fish was associated with hepatic necrosis (Marty et al. 1998). Meyers et al. (1994) and Marty et al. (1998) hypothesized that various combinations of stressors (inadequate food supply, spawning, other pathogens, or increased predation) resulted in activation of subclinical VHSV, which resulted in several lesions, including hepatic necrosis.

Our hypothesis is that sublethal concentrations of weathered oil can cause stress, resulting in immunosuppression, pathogen expression, and subsequent mortality in Pacific herring. This experiment was a serendipitous adjunct to other research; we had originally intended a disease-free reproduction experiment. Wild-caught Pacific herring in spawning condition were randomly separated into groups and exposed to petroleum hydrocarbons for 16–18 days: polynuclear aromatic hydrocarbon (PAH) concentrations were measured in treatment water, muscle, and ovaries. To document biochemical response, induction of mixed function oxidase (MFO) enzymes was observed in livers (Thomas et al. 1997). Total initial PAH (TPAH) concentrations in water (0.03 (controls) to 58 ppb) were chosen to include maximum mean TPAH concentrations observed in open water in PWS following the oil spill (6.24 ppb; Short and Harris 1996), and composition mimicked that observed in PWS.

Methods

Prespawning Pacific herring were collected near Shelter Island, southeast Alaska (58.4°N, 134.8°W), by purse seine on March 20, 1994. To minimize scale loss, fish were transferred in water and not netted. About 1500 fish were maintained in a 24 000-L holding tank; seawater flow was 60–130 L/min at 3.5–5.2°C and 31 ppt. Fish were not fed during holding or experimental periods. Gametes matured during holding, and fish were in spawning condition during experimental treatment.

An unknown percentage of Pacific herring developed signs of disease by April 1. Mortality (<3%) was associated with scale loss and raised white cutaneous foci near the dorsal fin. A Gram-negative filamentous rod resembling *Flexibacter* sp. was present, but no viral infections were detected in pooled liver and kidney tissues cultured from five fish. Fish were treated for bacterial infection beginning April 5: all fish received three successive 1-h static formalin baths (150 mg/L) at 2-day intervals. Mean holding mortality after treatment was 0.1–0.2%/day.

Seawater was oiled by contact with oiled rock; water flowed into a plenum at the bottom of 30-cm-diameter, 122-cm-long polyvinyl chloride plastic cylinders and upwelled through the rock. Water flowed from these oil generators to the bottom of individual treatment tanks; a trap inside the generator prevented slick overflow. Control generators were loaded with clean rock. To simulate the composition of oil stranded on PWS beaches in 1989 after the *Exxon Valdez* oil spill, the oil applied to gravel was artificially weathered by heating it to 70°C overnight until its initial mass was reduced by about 20% (Bence and Burns 1995). Gravel (median diameter 6.8 mm; range 2–33 mm) was washed on 3-mm screen and thoroughly air dried. A cement mixer was cleaned with soap and water and thoroughly air dried. Weathered crude oil, heated to 40°C, was applied with a paint sprayer to 45-kg batches of gravel tumbling in the cement mixer (trace-, low-, and mid-oil treatments) or with a Teflon squirt bottle (high-oil treatment). Batches of gravel were mixed by treatment; each generator was loaded with 45 kg of freshly oiled gravel (or nonoiled control rock), except high treatment generators, which were loaded with 90 kg of gravel.

There were five treatments, including control, with three replicates per treatment. Pacific herring without external lesions were randomly distributed among 15 700-L treatment tanks (50 fish per tank). Exposures lasted 16–18 days and began over a 3-day period for logistical reasons (April 24–26, 1994); fish were also sampled on day 8 for hydrocarbon measurement and a reproductive study (M.G. Carls, unpublished data). Water for each replicate tank flowed through an independent generator at 6.9 L/min and 4.2°C. To characterize the oil and quantify concentrations, composite samples (1.27 L from each of three replicate tanks) were collected and extracted on days 0, 8, and 16 for analysis by gas chromatography. Additional samples were simultaneously collected from each tank, extracted, and analyzed with ultraviolet spectroscopy (Krahn et al. 1991). Fish mortality and dissolved oxygen were monitored once per day in each tank. Tanks were located outside, but were protected from weather by a translucent shed roof. Fish ranged in size from 20.3 to 29.9 cm fork length and from 96 to 261 g wet weight; age ranged from 3 to 10 years. Mean age (6 years) did not vary significantly among tanks.

Analysis of PAH samples by gas chromatography – mass spectroscopy was performed at the Auke Bay Laboratory (Short et al. 1996). Experimentally determined method detection limits (MDL) depended on sample weights and generally were 1 ppb in tissue and 1–8 pptr (nanograms per litre) in water. Concentrations below MDL were treated as 0. The accuracy of the hydrocarbon analyses was about ±15% based on comparison with National Institute of Standards and Technology values, and precision expressed as coefficient of variation is usually less than about 20%, depending on the PAH. TPAH concentrations were calculated by summing concentrations of individual PAH. Tissue concentrations are reported on a wet weight basis.

After 16–18 days of exposure, fish were killed by a blow to the head or by anesthetization (tricaine methanesulfonate), measured (fork length to the nearest millimetre), weighed (wet weight to the nearest 0.01 g), and bled by clipping a gill arch. Livers were removed, frozen at –80°C, and later processed for MFO induction by Thomas et al. (1997); aryl hydrocarbon hydroxylase (AHH) activity was assayed according to the method of Nebert and Gelboin (1968). Gonad and muscle samples (10 g minimum) were collected with hydrocarbon-free dissection tools, frozen in hydrocarbon-free vials, and analyzed according to the methods of Short et al. (1996). Scales were collected near the posterior margin of the dorsal fin for age analysis. An additional 7–14 fish per replicate tank were sampled for viral erythrocytic necrosis (gill arches were clipped and blood smears were prepared), isolation of VHSV (anterior kidney and half of the spleen were frozen), and histopathology (gill, liver, and the other half of the spleen were preserved in 10% neutral buffered formalin) (Table 1). Virus isolation, in the EPC cell line, media formulation, and tissue preparation for cell line inoculation were performed as described by Meyers et al. (1994). VHSV was identified by DNA probe as described by Meyers et al. (1994). Additional necropsy data from all fish, including those that died before the test ended, included condition of skin, fins, eyes, jaw, gills, kidney, ovaries, spleen, and peritoneum. Percentages of fish with abnormal liver colors were determined; normal liver color

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Table 1. Number examined and length, weight, and VHSV prevalence in spawning Pacific herring after exposure to various concentrations of crude oil for 16–18 days in the laboratory.

	Treatment				
Variable	Control	Trace	Low	Mid	High
Total number of fish	31	30	32	30	30
Number of males	22	17	20	20	22
Mean fork length (mm) ± SE	228±3.9	232±3.3	229±2.9	227±3.3	232±3.4
Mean body weight (g) ± SE	119.6±0.20	124.8±0.19	113.8±0.16	108.8±0.15	128.0±0.14

was red. External bacterial infections were microscopically assessed from skin scrapings.

For histopathological processing and analysis, spleen, gill, and liver from 153 Pacific herring were assigned a code number known only to Alaska-based coauthors, and samples were shipped to the University of California, Davis. Tissues from each fish were mounted in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Sections from each organ were read blind in random order. Lesions were scored using a four-point scale as none (0), mild (1), moderate (2), or severe (3). For quality control, autolysis and artifact in each organ were scored on the same four-point scale. Ranking of lesions was often based on the number of inflammatory cells or organisms (e.g., resting spores of *Ichthyophonus hoferi*) per 100× or 400× field on a binocular light microscope. For example, hepatic perivascular and pericholangial eosinophilic granular leukocytes (EGL) per 400× field were scored as follows: 0 (≤2 EGL per field), 1 (>2 but ≤25 EGL per field), 2 (>25 EGL in at least one field and EGL extended to the margins of the surrounding parenchyma), or 3 (>25 EGL in at least one field and EGL extended into the surrounding parenchyma).

Data processing and statistics

Histopathological data were analyzed in two ways. First, overall significance of histopathology scores was determined using a multivariate analysis of variance (MANOVA). Histopathology scores were summarized through use of a principal components analysis. For a given set of scores, the first eight principal components based on the covariance matrix were used as a summary for most of the variability in the raw data. The principal components were then tested for systematic effects of oil exposure, VHSV status, length (as an estimate of age), and sex using MANOVA. Second, differences in VHSV and lesion prevalence were tested as functions of initial TPAH concentration with linear regression. Histopathological lesions categorized as artifact were not significantly related to oil treatment or VHSV in preliminary tests and were therefore not analyzed further. Five other lesions or parasites were removed from consideration because of insufficient response (ciliated protozoa, monogenetic or digenetic trematodes, and gill lamellar hyperplasia; $n_{\text{nonzero}} < 3$), total response (glycogen depletion; $n_{\text{nonzero}} = 153$), or near total response (macrophage aggregates in liver; $n_{\text{nonzero}} = 151$). Gall bladder myxosporeans were not analyzed due to incomplete data. Thus, only 25 of the original 38 lesion scores were analyzed. Stepwise regressions were used to compare the relative importance of TPAH and VHSV predictors. When regressions were significant, treatments were compared with controls with a priori multiple comparisons in single-factor ANOVA: percentage data were arcsin transformed and corrected for small n as necessary (Snedecor and Cochran 1980). Fish size and gender were eliminated as factors because they were never significant in tissues responsive to oil or VHSV in preliminary, nonparametric (Kruskal–Wallis) tests.

Results

Hydrocarbon exposure and accumulation

Initial aqueous TPAH concentrations ranged from 0.03 (control) to 58.3 ppb (Fig. 1). Concentrations in treatment water declined exponentially during the 16-day exposure period, but concentration declines were similar in all treatments; thus, treatment conditions remained clearly separable (Fig. 1). Initial aqueous alkane concentrations ranged from 1.28 (control) to 116 ppb, and concentration declines were similar to those of TPAH.

The relative abundances of aqueous PAH changed throughout the experiment. The predominant PAH initially present were the smaller and less substituted PAH (e.g., naphthalenes), but relative PAH abundances progressively shifted to larger and more substituted PAH (e.g., phenanthrenes) as smaller PAH were exhausted from the oiled gravel (Fig. 1). Smaller PAH were also exhausted from gravel more rapidly at progressively lower oil treatments.

PAH accumulated in muscle and ovaries of fish exposed to oil. In the high-oil treatment on day 16, TPAH concentrations in muscle tissue (24 038 ± 7347 ppb) significantly exceeded those in ovarian tissue (9688 ± 3551 ppb) ($P \leq 0.001$). Concentrations of TPAH in tissues after 16 days of exposure were correlated with initial TPAH concentrations ($0.92 \leq r \leq 0.99$, $P < 0.001$, where $y = (\text{TPAH in tissue})^{0.5}$).

Biological response

Mortality exceeded 10% in only one high-treatment tank during the first 8 days, but increased in the latter half of the experiment. Daily mortality was significantly related to time ($P = 0.011$) and treatment ($P < 0.001$), and cumulative mortality was significantly correlated with initial TPAH concentration ($r = 0.62$, $P = 0.013$) (Table 2; Fig. 2). Cumulative mortality was significantly greater in the mid-oil treatment than in controls, but variance in the mid-oil treatment was high and caused heterogeneous variance. However, when mid-oil responses were temporarily removed from the test to achieve homogeneous variance, the significance of mortality in the high-oil treatment was also apparent. Tank-specific differences in mortality, estimated by nesting tank in treatment, were not significant ($P = 0.672$).

Of the fish that died, 97% had lesions consistent with published descriptions of VHS in Pacific herring (Kocan et al. 1997; Marty et al. 1998), including external and internal hemorrhaging, fin erosion, changes in liver color, and gill pallor. The tissues of dead fish were not examined for pathogens, but development of lesions preceded death and matched lesions that were correlated with VHSV in living fish sampled at the end of study.

Gross lesions and other changes in living adult Pacific herring sampled at the end of the experiment were frequently correlated with initial TPAH concentration. Significantly and positively correlated with initial TPAH concentration were jaw hemorrhaging and erosion, changes

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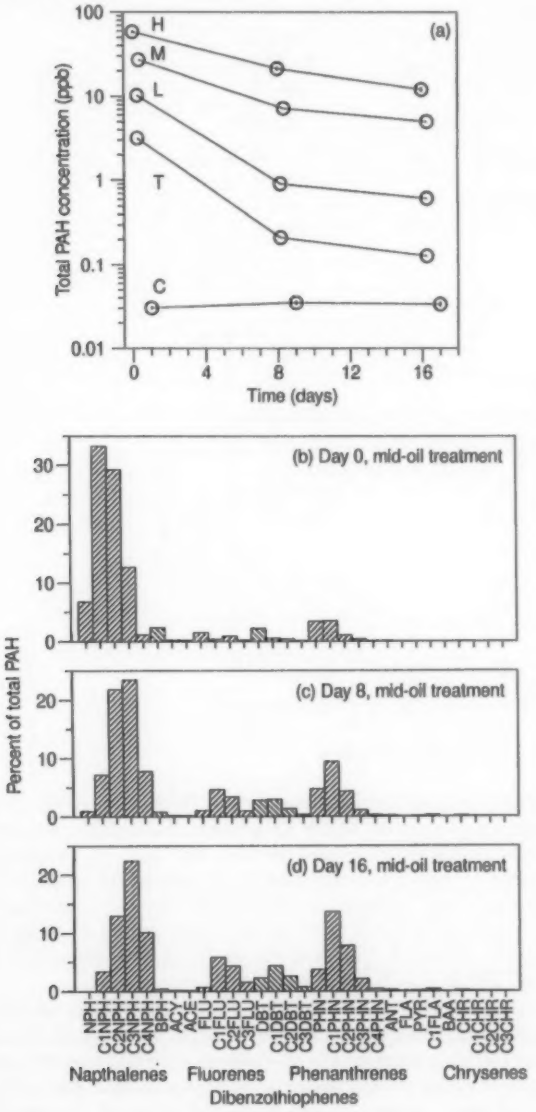
Fig. 1. TPAH concentrations in treatment water as a function of (*a*) time and (*b–d*) PAH composition. Data plotted are single composite values for replicate tanks at each time; standard error was estimated for the high-oil treatment from replicate fluorescence analyses (n = 3). Over time, composition shifted toward larger and more substituted PAH (e.g., phenanthrenes) as smaller PAH (e.g., naphthalenes) were exhausted from the oiled gravel. Treatments were control (C), trace (T), low (L), mid (M), and high (H) oil.



in liver color, and hemorrhaging of integument, peritoneum, and gonad (0. 64 ≤ r ≤ 0.80, 0.001 ≤ P_{reg} ≤ 0.010) (Fig. 3). Trends in fin erosion and gill pallor were similar, but not significant (P_{reg} = 0. 088 and 0.068, respectively). Prevalence of jaw lesions and nonnormal liver color was significantly elevated in mid- and high-oil treatments (P_{ANOVA} < 0.001) (Fig. 3). In contrast, spleen size and hemorrhaging of

Table 2. Correlation matrix showing relationships of major biological responses (AHH activity, EGL, VHSV, and cumulative mortality) and initial TPAH concentration in water.

	AHH	EGL	VHSV	Mortality
TPAH	0.52***	–0.84[a]***	0.79**	0.62*
AHH	—	–0.50***	0.52***	0.46***
EGL		—	–0.54[a]**	–0.70[b]**
VHSV			—	0.52[b]*

Note: Tabled values are correlation coefficients (r): n = 15 for all correlations not involving AHH, n = 116 for correlations involving AHH. Square root (x) transformations were applied unless otherwise noted. Asterisks indicate significance: ***$P \leq 0.001$, **$P \leq 0.01$, *$P \leq 0.05$.
[a]No transformation.
[b]ln(x) transformation.

eye and kidney did not vary as a function of oil treatment (0.375 ≤ P_{reg} ≤ 0.672). Correlation of gross lesions, or lack thereof, with prevalence of VHSV was similar to the correlation of gross lesions with TPAH, except that gonadal hemorrhaging did not correlate with VHSV (P = 0.290). Tested with stepwise regressions, TPAH was a better predictor of response than VHSV, except that hemorrhaging of peritoneum correlated more strongly with VHSV.

VHSV was detected in several fish collected at the end of the 16-day exposure (Fig. 2) whereas only one fish (of 140 examined) had erythrocyte inclusion bodies typical of viral erythrocytic necrosis, another pathogen commonly found in Pacific herring. Including all fish, mean VHSV titer generally increased from control to high-oil treatment, but was highest in the mid-oil treatment because one titer was two orders of magnitude greater than any other (titers ranged from 0 to 5 620 000). Restricted to VHSV-positive fish only, titer tended to decline with increasing treatment, but was greatest in the mid-oil treatment. Due to high variance, titer differences among treatments were not significant ($P \geq$ 0.260). However, prevalence of VHSV (Fig. 2) was correlated with initial TPAH concentration (r = 0.78, P = 0.001) (Table 2) and was significantly greater in high-oil treatment fish than in controls (Fig. 2).

Differences in lesion scores among treatment groups were highly significant with respect to hydrocarbon concentration and VHSV (MANOVA, Wilks' lambda, P < 0.001). Prevalences of several histopathological lesions were correlated with TPAH concentration or VHSV prevalence. Significant lesions, listed from most to least significant P value, included EGL, splenic thrombosis, gill arch inflammation or hematopoiesis, focal parenchymal leukocytes in the liver, branchial *Epitheliocystis*, single-cell hepatocellular necrosis or apoptosis, hepatic focal necrosis, foreign body granuloma, and splenic arteriolar focal intimal hyperplasia (Table 3; Figs. 2 and 4). Conservatively, the overall error rate for multiple, independent analyses could be controlled by dividing α (0.05) by the number of tests (25); only EGL and splenic thrombosis passed this criterion. Gill arch inflammation or hematopoiesis declined as TPAH concentration increased (r = –0.68, P = 0.005) and with prevalence of VHSV (r = –0.73, P = 0.002). Differences in length and gender were not significant for any comparisons (MANOVA).

Focal hepatic necrosis and disseminated vascular thrombosis were among the most severe lesions associated with oil level and VHSV. Hepatic necrosis usually affected multi-

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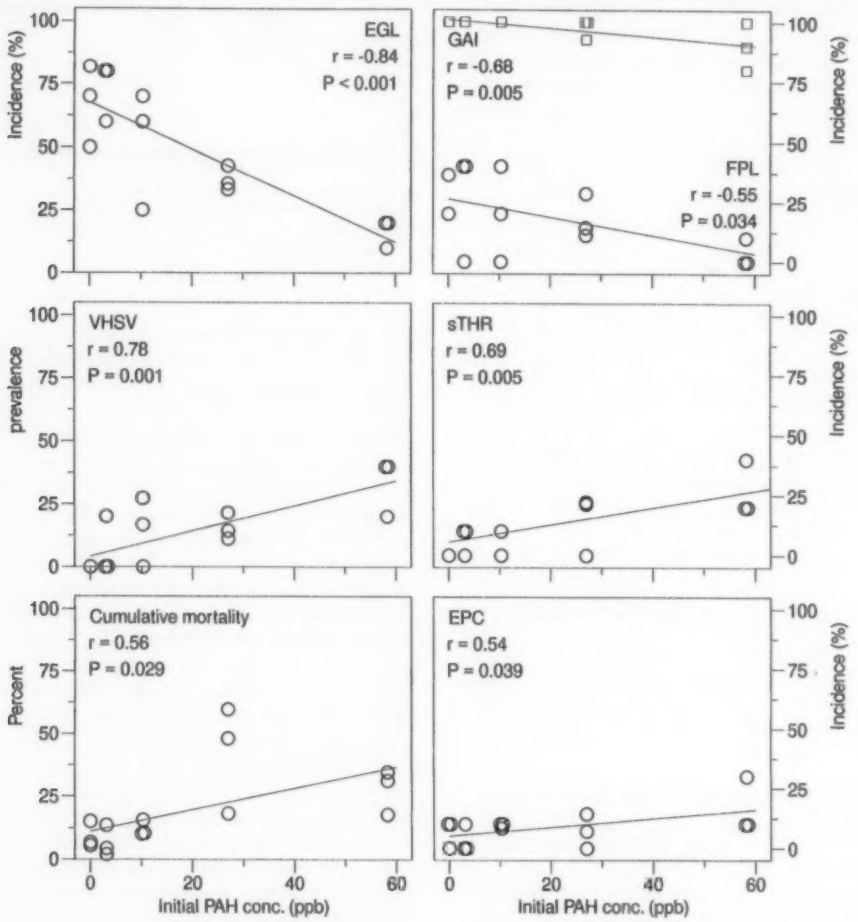
Fig. 2. Relationship of cumulative mortality and significant histopathological responses to initial TPAH concentration. Percentages of EGL, VHSV, gill arch inflammation (GAI), focal/multifocal parenchymal leukocytes (FPL), splenic thrombosis (sTHR), and *Epitheliocystis* (EPC) were regressed against TPAH: *P* is probability of significant slope and *r* is correlation coefficient.



ple random foci throughout the parenchyma (Fig. 4). Affected hepatocytes had hypereosinophilic cytoplasm with pyknotic or karyorrhectic nuclei. Of the six affected fish, one was a control but the other five were from the three highest dose groups. No fish had severe hepatic necrosis, but the two moderate cases were in the two highest exposure groups. Four of the six fish with hepatic necrosis were positive for VHSV; among the two moderate cases, one was negative for VHSV.

Vascular thrombosis was more common, occurred in all three organs, and affected 29 of 153 fish (19%). Only one affected fish was a control. Thrombus composition varied from cellular to fibrinous. Cellular thrombi, usually <100 μm in diameter, were composed of clusters of thrombocytes and scattered macrophages, with comparatively little extracellular material; cellular thrombi were never occlusive. Fibrinous thrombi were usually larger, sometimes >1 mm in diameter and occlusive, but thrombocytes were rare (Fig. 4). Prevalence of thrombi increased with oil treatment in spleens and possibly gills. Splenic thrombosis increased as TPAH concentration increased (r = 0.79, P = 0.001) and with prevalence of VHSV (r = 0.75, P = 0.001); thrombi were significantly elevated in mid- and high-oil treatments (Table 3; Fig. 2). Gill thrombosis was rare (eight nonzero responses), and correlation with TPAH was not significant (r = 0.40, P = 0.141), but trends were similar to those in spleens (Table 3). Thrombosis in livers did not correlate with TPAH (r = 0.13, P = 0.654).

Abundance of tissue leukocytes was depressed as oil concentration increased. Incidence of EGL declined as TPAH concentration increased (r = –0.84, P < 0.001) and with increased VHSV prevalence (r = –0.54, P = 0.038); EGL were significantly depressed in mid- and high-oil treatments (Fig. 2). Prevalence of EGL was also negatively correlated with AHH activity (r = –0.50, P < 0.001) reported in a companion study (Thomas et al. 1997). Focal or multifocal parenchymal leukocytes were also inversely correlated with TPAH concentration (r = –0.55, P = 0.034), but not with VHSV (r = –0.22, P = 0.422) (Table 3). In contrast, pericholangial leukocytes did not correlate with either TPAH

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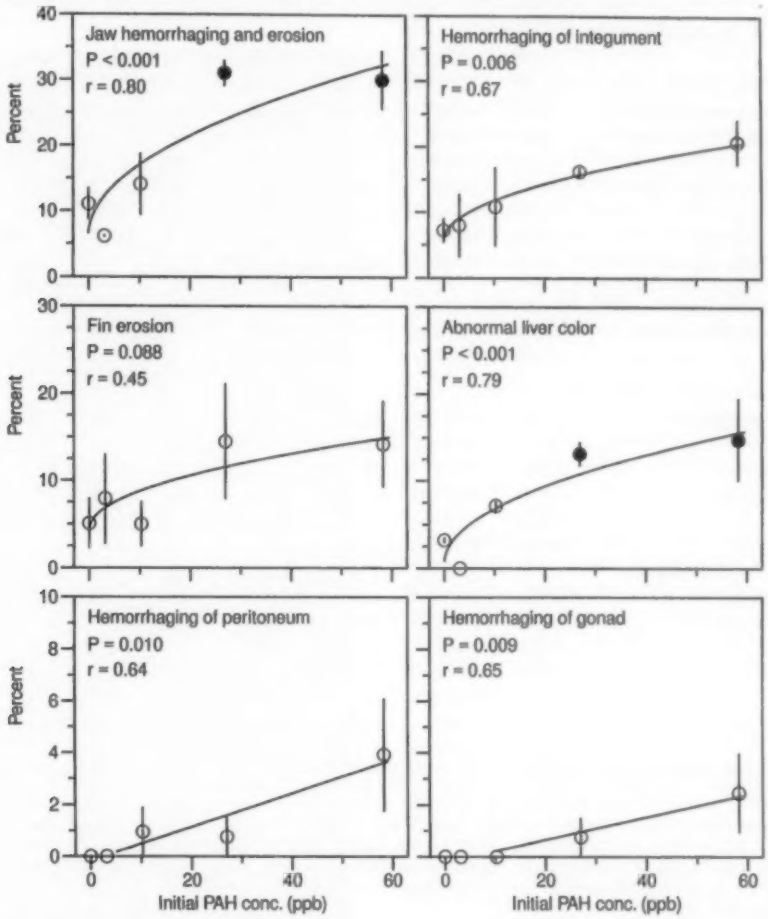
Fig. 3. Gross lesions (mean ± SE) in Pacific herring that significantly correlated with initial TPAH concentration. Control (normal) liver color was red. Percentages were regressed against TPAH or $TPAH^{0.5}$: *P* is probability of significant slope and *r* is correlation coefficient. Solid symbols indicate significant differences from controls, determined by ANOVA.



(r = –0.30, P = 0.271) or VHSV (r = –0.39, P = 0.153), although trends were similar to those of other leukocytes (Table 3).

The paucity of inflammatory cells in Pacific herring exposed to the highest dose of oil was particularly obvious. Among the five treatment groups, organs from fish exposed to the highest dose of oil had significantly lower mean scores for several inflammatory lesions: (*i*) hepatic perivascular or pericholangial EGL, (*ii*) gill arch inflammation or hematopoiesis, (*iii*) focal or multifocal hepatic parenchymal leukocytes, and (*iv*) granulomatous inflammation in the spleen. For example, 21 of 31 livers from control fish had pericholangial or perivascular EGL whereas only five of 30 livers from the high-dose group had EGL. EGL were nearly always scored as none or mild; the two moderate cases were in the control and lowest dose groups. Mean scores for other inflammatory lesions were lower in the high-dose fish, but trends were not significant: (*i*) *Ichthyophonus* in the spleen and liver, (*ii*) cholangitis or biliary hyperplasia, and (*iii*) intrahepatic pericholangial leukocytes.

Lesions caused by parasites were not related to oil exposure or VHSV. Overall prevalences of various parasites were as follows: *Ichthyophonus hoferi*, 11%; branchial *Epitheliocystis*, 8.6%; hepatic *Goussia clupearum*, 81%; hepatic Anisakidae, 16%.

Discussion

Exposure of wild Pacific herring to crude oil in this preliminary experiment was associated with expression of VHSV and mortality, and VHSV expression may have resulted from immunosuppression. Histological lesions in these fish could have resulted either directly from the presence of hydrocarbons in tissues or secondarily from VHSV. Concentrations of axenic hydrocarbons were elevated in tissues of fish exposed to oil, and MFO enzymes were induced (Thomas et al.1997). Thus, it is possible that organ, tissue, or cellular damage was caused directly by hydrocarbons. More likely, however, certain histological lesions were caused by expression of VHSV. Alternatively, the lesions

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Table 3. Relationship of histological lesion prevalences to oil and VHSV and mean lesion scores.

	Oil regression		VHSV regression			Mean lesion scores				
	r	P	r	P	*n*	C	T	L	M	H
Lesions responsive to oil or VHSV										
Thrombosis, spleen	0.79	**0.001**[a]	0.75	**0.001**	16	0.00	0.07	0.06	0.17	0.50
Leukocytes, eosinophilic granular, liver	–0.84	**0.001**[a]	–0.54	**0.038**	75	0.71	0.77	0.50	0.37	0.17
Gill arch inflammation/hematopoiesis	–0.68	**0.005**	–0.73	**0.002**[a]	149	1.10	1.00	1.03	1.07	0.93
Leukocytes, focal or multifocal, liver	–0.55	**0.034**[a]	–0.22	0.422	28	0.26	0.27	0.19	0.17	0.03
Epitheliocystis, gill	0.53	**0.040**[a]	0.51	0.051	13	0.10	0.03	0.09	0.07	0.17
Hepatocellular necrosis, single cell	0.47	0.077	0.65	**0.008**[a]	22	0.13	0.17	0.13	0.27	0.23
Hepatic necrosis, focal	0.34	0.146[b]	0.56	**0.028**[a]	6	0.03	0.00	0.06	0.07	0.10
Foreign body granuloma, gill	–0.45	0.096	–0.54	**0.036**[a]	11	0.06	0.13	0.09	0.07	0.00
Focal intimal hyperplasia, spleen	–0.45	0.090	–0.53	**0.041**[a]	19	0.13	0.20	0.09	0.17	0.03
Lesions possibly responsive to oil or VHSV ($P \leq 0.15$)										
Granulomatous inflammation, spleen	–0.51	0.052[a]	–0.20	0.482	10	0.06	0.17	0.09	0.07	0.00
Thrombosis, gill	0.40	0.141[a]	0.15	0.597	8	0.03	0.00	0.03	0.20	0.13
Lesions not related to oil or VHSV ($P > 0.15$)										
Macrophage aggregates, spleen	0.37	0.175	0.35	0.196	138	1.29	1.60	1.22	1.40	1.37
Hepatic lipidosis	0.37	0.175	0.04	0.877	16	0.13	0.10	0.16	0.13	0.23
Gill lamellar telangiectasis	0.03	0.270	0.13	0.656	62	0.16	0.67	0.44	0.67	0.60
Leukocytes, pericholangial	–0.30	0.271	–0.39	0.153	25	0.13	0.30	0.13	0.17	0.10
Ichthyophonus, liver	–0.26	0.355	–0.17	0.534	12	0.06	0.37	0.09	0.23	0.03
Goussia clupearum, liver	0.25	0.360	0.01	0.981	124	1.35	1.17	1.16	1.00	1.07
Cholangitis or biliary hyperplasia	–0.25	0.375	–0.15	0.592	8	0.13	0.07	0.06	0.03	0.03
Granulomatous inflammation, liver	–0.23	0.400	–0.32	0.240	35	0.26	0.53	0.25	0.17	0.23
Ellipsoid hyalinization, spleen	–0.21	0.461	0.03	0.906	142	1.16	1.23	1.13	1.17	1.27
Anisakis, liver	–0.21	0.468	–0.03	0.903	24	0.13	0.23	0.28	0.10	0.13
Ichthyophonus, spleen	–0.16	0.570	–0.22	0.441	12	0.10	0.23	0.13	0.20	0.07
Thrombosis, liver	0.13	0.654	0.01	0.967	7	0.00	0.03	0.09	0.17	0.03
Ichthyophonus, gill	–0.07	0.797	–0.21	0.454	9	0.06	0.13	0.03	0.03	0.07
Congestion, spleen	0.05	0.871	0.16	0.565	139	1.61	1.47	1.13	1.27	1.50

Note: *r* is correlation coefficient, *P* is probability, and *n* is the total number of nonzero lesion scores of 153 possible. Lesions were grouped into three categories, based on response or lack of response to oil treatment or VHSV. The responsive category included lesions where P_{oil} or $P_{VHSV} \leq 0.05$. The "possibly responsive" category included lesions where $P \leq 0.15$. Lesions where $P > 0.15$ were considered unrelated to oil treatment. Bold type indicates statistical significance ($P \leq 0.05$). In the one instance where both TPAH and VHSV entered the regression (focal hepatic necrosis), final model correlations and probabilities are reported; in all other cases, reported values indicate separate regressions against TPAH or VHSV. C, control; T, trace-oil treatment; L, low-oil treatment; M, mid-oil treatment; H, high-oil treatment.

[a]Factor that entered the stepwise regression first.

[b]Factor that entered the stepwise regression second.

may have been caused by a combination of oil exposure and VHSV expression.

Concentrations of oil to which Pacific herring were exposed were considered to be environmentally relevant. Conservatively, we analyzed biological responses of Pacific herring against initial TPAH exposure concentrations, which bracketed, but extended well beyond, those reported in open water of PWS after the spill (up to 6.24 ppb; Short and Harris 1996). However, because test concentrations fell rapidly with time, effective exposure concentrations were lower. For example, mean TPAH concentrations in low- and mid-oil treatments (4 and 13 ppb) bracketed the 6-ppb value, and significant biological response was observed in this range. In PWS, TPAH concentrations were also variable and generally declined with time. It is possible, depending on the volume of stranded oil and other factors, that concentrations were higher in intertidal areas and that significant concentrations persisted longer intertidally than in open water. For these reasons, it is plausible that some reproductively ripe Pacific herring could have encountered biologically significant quantities of oil during intertidal spawning activity or as they migrated through the slick trajectory. Observed PAH composition and weathering were very similar to those of *Exxon Valdez* crude oil in PWS; weathering could be explained by first-order loss-rate kinetics (Short and Heintz 1997).

Fish are frequently asymptomatic carriers of pathogens that are controlled by their immune system (Anderson 1990), and Pacific herring are reservoirs of VHSV (Meyers et al. 1994). Preliminary estimates are that 10–20% of wild Pacific herring carry VHSV below detectable levels (G.D. Marty and R.M. Kocan (School of Fisheries, University of Washington, Box 355100, Seattle, WA 98195, U.S.A.), unpublished data). This explanation was likely true for our fish prior to the experiment. We do not know whether the virus in our fish was nonreplicating or replicating but not detectable, but we refer to nondeductible cases as "subclinical." That the virus was not isolated from control fish indicated

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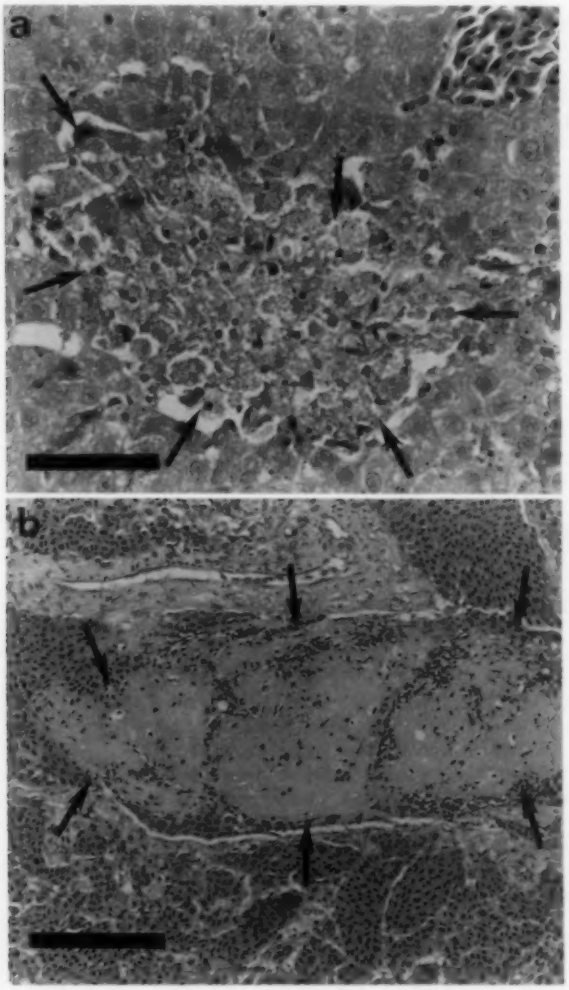
Fig. 4. Microscopic lesions in adult Pacific herring exposed to weathered crude oil in the laboratory. (*a*) Moderate focal hepatocellular necrosis (arrows); scale bar = 40 μm. (*b*) Fibrinous thrombi (arrows) in a congested spleen; scale bar = 100 μm.



that exposure to VHSV through the common water supply was unlikely in our experiments.

We hypothesize that stress followed by decreased immune surveillance is a mechanism by which subclinical VHSV could be reactivated. Because a variety of toxicants have been shown to compromise the immune system (Anderson 1990), decreased inflammation was more likely a nonspecific response to stress than a specific response to VHSV or oil exposure. Several laboratory studies have documented the relationship between stress and disease expression. For example, the titer of infectious pancreatic necrosis virus was elevated in brook trout (*Salvelinus fontinalis*) treated with immunosuppressant and stressed with warm water (McAllister et al. 1994). Susceptibility of rainbow trout (*Oncorhynchus mykiss*) to infectious hematopoietic necrosis virus increased when they were exposed to copper (Hetrick et al. 1979). Also, brook trout known to carry *Aeromonas salmonicida* developed disease signs after injection of a synthetic corticosteroid (Bullock and Stuckey 1975). Examination of Pacific herring tissues after exposure to other stressors such as crowding or altered water temperature is needed to confirm the role of stress in intrahepatic inflammation and VHSV expression.

Decreased prevalence of several types of inflammatory cells in livers as oil concentration increased was evidence for generalized immunosuppression in exposed Pacific herring. Decreased intrahepatic inflammatory cells in response to oil exposure have not previously been described. This lack of published information may not be due to lack of occurrence; rather, diagnosis of decreased inflammatory cells may have been missed. In our experience, small numbers of inflammatory cells are normal in the liver of adult Pacific herring (Kocan et al. 1996; Marty et al. 1998).

Reproductive condition in our study probably was a contributing stress factor because gamete production requires considerable energy (gonad mass exceeded 20% of total body weight) and because of limited natural food availability (only 6% of the captured prespawning fish in our study had food in their gastrointestinal tract). As evidence, intensive biannual sampling (1993–1998) of Pacific herring in Prince William Sound, Alaska, yielded VHSV-positive samples (prevalence up to 15%) in prespawning and spawning fish in all years except 1996 (Meyers and Winton 1995; Marty et al. 1998; T.R. Meyers and G.D. Marty, unpublished data). By comparison, VHSV has never been isolated from fish sampled in the fall.

Results from published studies support the hypothesis that hepatic necrosis was more a result of VHSV than oil exposure. Lesions commonly associated with oil include decreased hepatocellular glycogen and variable alterations in hepatocellular lipid (Hawkes 1977; McCain et al. 1978; Eurell and Haensly 1981; Haensly et al. 1982; Solangi and Overstreet 1982; Woodward et al. 1983). Although hepatocellular necrosis has been associated with environmental oil exposure (Haensly et al. 1982), this finding has not been confirmed in the laboratory. By comparison, hepatic necrosis is common in salmonids with the European strain of VHSV (Amlacher et al. 1980; Wolf 1988).

Thrombosis has not been described in association with either oil exposure or VHSV. Deficiency of thrombocytes and leukocytes and anemia have been associated with the European strain of VHSV in salmonids (Wolf 1988). In contrast, a relationship between TPAH concentration and gill-vessel aneurysms that could lead to thrombosis has been observed in Pacific sanddab (*Citharichthys sordidus*) (R.B. Spies, Applied Marine Sciences, 2155 Las Positas Court, Suite S, Livermore, CA 94550, U.S.A., personal communication).

Expression of VHSV apparently caused mortality in the laboratory, and may also cause mortality in wild populations. Moribund Pacific herring were observed in PWS in 1993, and pooled samples that included these fish had VHSV (Meyers et al. 1994). Although it was not possible to quantify mortality caused by disease, only one third of the expected spawning biomass returned in 1993 (Meyers et al. 1994). In contrast, postspawning Pacific herring similarly

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tested in a companion study (Thomas et al. 1997) did not develop clinical signs of disease, and mortality related to oil dose did not occur. This suggests that mortality in prespawning fish was related to disease, and possibly reproductive or nutritional condition, but not to direct toxic effects. Research to determine if the toxic effects of environmentally relevant concentrations of crude oil on Pacific herring are entirely a result of VHSV expression are underway (R.M. Kocan, School of Fisheries, University of Washington, Box 355100, Seattle, WA 98195, U.S.A., personal communication).

Although effects due to oil and VHSV effects were not definitively separable in our study, we suggest that gross and histological lesions previously attributed solely to oil exposure in Pacific herring captured from Prince William Sound in 1989 (Moles et al. 1993) may have been caused by expression of VHSV, induced by oil exposure. This suggestion is further supported by occurrence of similar lesions in VHSV-positive Pacific herring sampled from Prince William Sound in 1993, 1994, and 1997, when hydrocarbon exposure was no longer detectable (Meyers et al. 1994; Marty et al. 1998; G.D. Marty, unpublished data). Hepatic necrosis has been confirmed in disease-free juvenile Pacific herring that were exposed to VHSV but not oil (Kocan et al. 1997).

In summary, correlations between oil exposure, induction of AHH, suppression of leukocytes, prevalence of VHSV, and cumulative mortality were strong, suggesting possible causal links between oil exposure and immunosuppression, between immunosuppression and expression of VHSV, and between VHSV and mortality in Pacific herring exposed to oil. Results of this study do not prove causality, but they do show that hydrocarbon exposure, decreased tissue leukocytes, disease, and mortality are closely related.

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References

Amlacher, E., Ude, J., Rudolph, C., and Ernst, G. 1980. Direct electron microscopical visualization of the presumptive virus of viral haemorrhagic septicaemia (VHS) in the rainbow trout *Salmo gairdneri* Richardson and additional histopathological and haematological observations. J. Fish Dis. **3**: 55–62.

Anderson, D.P. 1990. Immunological indicators: effects of environmental stress on immune protection and disease outbreaks. Am. Fish. Soc. Symp. **8**: 38–50.

Bence, A.E., and Burns, W.A. 1995. Fingerprinting hydrocarbons in the biological resources of the *Exxon Valdez* spill area. *In Exxon Valdez* oil spill: fate and effects in Alaskan Waters. *Edited by* P.G. Wells, J.N. Butler, and J.S. Hughes. American Society for Testing and Materials, Philadelphia, Pa. pp 84–140.

Bullock, G.L., and Stuckey, H.M. 1975. *Aeromonas salmonicida*: detection of asymptomatically infected trout. Prog. Fish-Cult. **37**: 237–239.

Eurell, J.A.C., and Haensly, W.E. 1981. The effects of exposure to water soluble fractions of crude oil on selected histochemical parameters of the liver of the Atlantic croaker, *Micropogon undulatus*. L. J. Fish Dis. **4**: 187–194.

Haensly, W.E., Neff, J.M, Sharp, J.R, Morris, A.C., Bedgood, M.F., and Boem, P.D. 1982. Histopathology of *Pleuronectes platessa* L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the *Amoco Cadiz* crude oil spill. J. Fish Dis. **5**: 365–391.

Hawkes, J.W. 1977. The effects of petroleum hydrocarbon exposure on the structure of fish tissues. *In* Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems, Proceedings. *Edited by* D.A. Wolfe. Pergamon Press, Elmsford, N.Y. pp. 115–128.

Hetrick, F.M., Knittel, M.D., and Fryer, J.L. 1979. Increased susceptibility of rainbow trout to infectious hematopoietic necrosis virus after exposure to copper. Appl. Environ. Microbiol. **37**: 198–201.

Khan, R.A. 1990. Parasitism in marine fish after chromic exposure to petroleum hydrocarbons in the laboratory and to the *Exxon Valdez* oil spill. Bull. Environ. Contam. Toxicol. **44**: 759–763.

Khan, R.A., and Kiceniuk, J. 1983. Effects of crude oils on the gastrointestinal parasites of two species of marine fish. J. Wildl. Dis. **19**: 253–258.

Kocan, R.M., Marty, G.D., Okihiro, M.S., Brown, E.D., and Baker, T.T. 1996. Reproductive success and histopathology of individual Prince William Sound herring 3 years after the *Exxon Valdez* oil spill. Can. J. Fish. Aquat. Sci. **53**: 2388–2393.

Kocan, R.M., Bradley, M., Elder, N., Meyers, T., Batts W., and Winton, J. 1997. The North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory reared Pacific herring. J. Aquat. Anim. Health, **9**: 279–290.

Krahn, M.M., Ylitalo, G.M., Joss, J., and Chan, S.-L. 1991. Rapid, semi-quantitative screening of sediments for aromatic compounds using sonic extraction and HPLC/fluorescence analysis. Mar. Environ. Res. **31**: 175–196.

Malins, D.C. 1982. Alterations in the cellular and subcellular structure of marine teleosts and invertebrates exposed to petroleum in the laboratory and field: a critical review. Can. J. Fish. Aquat. Sci. **39**: 877–889.

Marty, G.D., Freiberg, E.F., Meyers, T.R., Wilcock, J., Farver, T.B., and Hinton, D.E. 1998. Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* spawning in Prince William Sound, Alaska, USA. Dis. Aquat. Org. **32**: 15–40.

McAllister, P.E., Owens, W.J., and Schill, W.B. 1994. Effects of elevated water temperature and immunosuppressant treatment on prevalence and titer of infectious pancreatic necrosis in naturally infected brook trout. J. Aquat. Anim. Health, **6**: 133–137.

McCain, B.B., Hodgins, H.O., Gronlund, W.D., Hawkes, J.W., Brown, D.W., Myers, M.S., and Vandermeulen, J.H. 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole (*Parophrys vetulus*), and pathological consequences. J. Fish. Res. Board Can. **35**: 657–664.

Meyers, T.R., and Winton, J.R. 1995. Viral hemorrhagic septicemia virus in North America. Annu. Rev. Fish Dis. **5**: 3–24.

Meyers, T.R., Short, S., Lipson, K., Batts, W.N., Winton, J.R., Wilcock, J., and Brown, E. 1994. Association of viral hemor-

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rhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA. Dis. Aquat. Org. **19**: 27–37.

Moles, A.D., Rice, S.D., and Okihiro, M.S. 1993. Herring parasite and tissue alterations following the *Exxon Valdez* oil spill. *In* Proceedings of the 1993 Oil Spill Conference, American Petroleum Institute Publ. No. 4580. American Petroleum Institute, Washington, D.C. pp. 325–328.

Nebert, D.W., and Gelboin, H.V. 1968. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. J. Biol. Chem. **243**: 6242–6249.

Short, J.W., and Harris, P.M. 1996. Chemical sampling and analysis of petroleum hydrocarbons in near-surface seawater of Prince William Sound after the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. **18**: 17–28.

Short, J.W., and Heintz, R.A. 1997. Identification of *Exxon Valdez* oil in sediments and tissues of PWS. Environ. Sci. Technol. **31**: 2375–2384.

Short, J.W., Jackson, T.J., Larsen, M.L., and Wade, T.L. 1996. Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resources damage assessment of the *Exxon Valdez* oil spill. Am. Fish. Soc. Symp. **18**: 140–148.

Sindermann, C.J. 1993. Interactions of pollutants and disease in marine fish and shellfish. *In*. Pathobiology of marine and estuarine organisms. *Edited by* J.A. Couch and J.W. Fournie. CRC Press, Boca Raton, Fla. pp. 451–482.

Snedecor, G.W., and Cochran, W.G. 1980. Statistical methods. 7th ed. Iowa State University Press, Ames, Iowa.

Solangi, M.A., and Overstreet, R.M. 1982. Histopathological changes in two estuarine fishes, *Menidia beryllina* (Cope) and *Trinectes maculatus* (Bloch and Schneider), exposed to crude oil and its water soluble fractions. J. Fish Dis. **5**: 13–35.

Thomas, R.E., Carls, M.G., Rice, S.D., and Shagrun, L. 1997. Mixed function oxidase induction in pre- and post-spawn herring (*Clupea pallasi*) by petroleum hydrocarbons. Comp. Biochem. Physiol. C Comp. Pharmacol. Toxicol. **116**: 141–147.

Wolf, K. 1988. Fish viruses and fish viral diseases. Cornell University Press, Ithaca, N.Y.

Woodward, D.F., Riley, R.G., and Smith, C.E. 1983. Accumulation, sublethal effects, and safe concentration of a refined oil as evaluated with cutthroat trout. Arch. Environ. Contam. Toxicol. **12**: 455–464.

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High rate of redd superimposition by brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) in a Minnesota stream cannot be explained by habitat availability alone

Timothy E. Essington, Peter W. Sorensen, and Dean G. Paron

Abstract: This study was conducted to test the hypothesis that redd superimposition by salmonine fishes is a consequence of limited habitat availability. We monitored redd site selection by brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for two spawning seasons in Valley Creek, Minnesota. Redd superimposition rates were high; over one half of the brook trout and one third of the brown trout superimposed redds. We tested the role of habitat availability in this process by characterizing microhabitat at sites with and without redds in four small sections of this stream and then determined whether superimposition could be explained by random dispersal of fish over available habitat. Brown trout preferred spawning sites with high flows whereas brook trout strongly preferred deep sites with upwelling groundwater. No relationship was observed between fish density and superimposition. Additionally, the observed frequency of superimposition was greater than expected by chance in six of eight instances for brown trout and in one of three instances for brook trout. Finally, a behavioral experiment provided direct evidence that females have a behavioral preference to spawn on existing redd sites, suggesting that factors other than habitat may determine redd site selection and hence superimposition.

Résumé : La présente étude visait à tester l'hypothèse selon laquelle la superposition des nids de salmoninés découlerait de la faible disponibilité de l'habitat. Nous avons surveillé les nids de l'omble de fontaine (*Salvelinus fontinalis*) et de la truite brune (*Salmo trutta*) pendant deux saisons de fraye dans le crique Valley, au Minnesota. Le taux de superposition des nids était élevé; nous avons observé ce comportement chez plus de la moitié des ombles de fontaine et le tiers des truites brunes. Nous avons vérifié le rôle de la disponibilité de l'habitat dans ce processus en caractérisant le microhabitat aux sites avec ou sans nids dans quatre petits tronçons de ce cours d'eau, puis nous avons déterminé si la superposition pouvait être expliquée par la dispersion au hasard des poissons dans l'ensemble de l'habitat disponible. Les truites brunes préfèrent les sites de fraye à débit élevé tandis que l'omble de fontaine préfèrent nettement les sites profonds à remontée d'eau souterraine. Aucune relation n'a été observée entre la densité des poissons et la superposition des nids. En outre, la fréquence de superposition observée était supérieure à celle prévue en fonction du hasard dans six des huit cas chez la truite brune et dans un des trois cas chez l'omble de fontaine. En fin de compte, une expérience comportementale a montré directement que les femelles préfèrent pondre dans des nids existants, ce qui laisse supposer que des facteurs autres que l'habitat peuvent présider au choix des nids et donc être à l'origine de la superposition.

[Traduit par la Rédaction]

Introduction

Reuse of redd sites by later-spawning fish (redd superimposition) has been frequently documented in salmonine fishes (McNeil 1964, 1966; Tautz and Groot 1975; Witzel and MacCrimmon 1983; Keenleyside and Dupuis 1988; Curry and Noakes 1995; Sorensen et al. 1995). This behavior removes eggs previously placed in redds and is an important factor influencing the reproductive success of salmonine fishes (Hayes 1987; van den Berghe and Gross 1989). For example, Hayes (1987) found that the reproductive success of brown trout (*Salmo trutta*) in a small New Zealand tributary was reduced over 94% due to redd superimposition by later-spawning rainbow trout (*Oncorhynchus mykiss*). Further, van den Berghe and Gross (1989) suggested that reproductive losses associated with redd superimposition play an important role in determining the spawning date of female coho salmon (*Oncorhynchus kisutch*).

Despite the frequency of its occurrence and the clear potential for redd superimposition to affect reproductive success, the causes of redd superimposition have not been systematically evaluated. Redd superimposition is frequently attributed to the limited availability of spawning habitat

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(e.g., Ligon et al. 1995), perhaps because this behavior has been extensively documented in anadromous salmonine species which often face severe habitat limitations (e.g., McNeil 1964, 1966; Fleming and Gross 1989; van den Berghe and Gross 1989). However, redd superimposition has also been reported in stream-resident salmonine species, which typically do not spawn at such high densities (Witzel and MacCrimmon 1983; Beard and Carline 1991; Sorensen et al. 1995). These observations suggest that redd superimposition may be the result of a behavioral preference to spawn on existing redds rather than the result of competition for limited space (Witzel and MacCrimmon 1983). However, no one has directly tested these alternative explanations.

The goal of this study was to test these hypotheses using data collected on spawning brown trout and brook trout (*Salvelinus fontinalis*) in a small stream where habitat appeared abundant yet redd superimposition was common (Sorensen et al. 1995). We described the frequency of redd superimposition, identified available habitat using a novel multivariate procedure, and determined whether habitat availability could explain the observed frequency of superimposition. Finally, we conducted a behavioral experiment to directly evaluate females' preference for existing redd sites.

Materials and methods

Study site

Valley Creek is a low-gradient, second-order tributary of the St. Croix River, located about 30 km east of St. Paul, Minn. Valley Creek is ideal for behavioral observations of trout due to its small size (mean width about 4 m and mean depth about 0.2 m) and relative lack of surface turbulence at spawning sites (Sorensen et al. 1995). In this study, we selected two 200-m reaches of the stream in which to monitor the distribution of spawning females. These reaches had consistently high densities of spawning fish in the past (Sorensen et al. 1995) and were readily accessible by foot and easily observed. Section 1 (surface area = 920 m^2) contained only brown trout whereas section 2 (surface area = 850 m^2) contained both brook and brown trout.

Describing the frequency of redd superimposition

We monitored the spawning activity in both study sections two or three times daily from 29 September to 23 November in 1993 and from 1 October to 25 November in 1994 (when most spawning occurs; Sorensen et al. 1995). Redds sites were mapped and individual redds were considered to be superimposed if they overlapped with another by more than 50%. When female spawning behavior was observed on the same redd for consecutive days, we considered this to be a single female unless it was apparent based on the size of females or their species that two females were using the same redd site on consecutive days. If females could not be distinguished, we considered redds to be superimposed only if 2 or more days had passed between observations of reproductive activity on redd sites. This assumption was based on a 1993 study in which only one of 33 tagged brook and brown trout individuals returned to a redd after being inactive for 2 or more days (Essington 1995).

Characterization of microhabitat

We characterized the microhabitat of all redd sites and 50 randomly selected sites within four subsections located within the study reaches. Subsections ranged in size from 17 to 57 m^2 and were selected because they contained relatively homogeneous, high-quality spawning habitat and were frequently used by spawning trout. Two subsections were located within each section. Random sites were selected by generating pairs of random numbers that denoted the latitudinal and longitudinal position relative to a fixed reference site. Six habitat parameters were measured at each site: depth, mean water column velocity, bottom water velocity, groundwater upwelling, substrate composition, and substrate embeddedness. This list included all parameters previously suggested to be important in redd site selection in these species (Hansen 1975; Shirvell and Dungey 1983; Witzel and MacCrimmon 1983; Curry and Noakes 1995; Sorensen et al. 1995). Water velocity was measured with a Marsh McBirney digital flowmeter; mean water column velocity was measured at 60% of the water depth, and bottom velocity was measured 3 cm off the bottom. We estimated the proportion of groundwater within the substrate by measuring the temperature difference between water within the substrate and water in the stream channel just below the water surface (Sorensen et al. 1995). The range of temperature differences between stream and groundwater (0.0–4.0°C) exceeded the precision of our technique (0.5°C). Substrate was visually assessed at each site by estimating the percent composition of the following size classes: small (silt to 3 mm), medium (3–20 mm), and large (>20 mm). We classified substrate embeddedness according to whether the substrate could be dislodged with a small garden shovel with (1) little effort, (2) moderate effort, or (3) considerable effort. The latter method was intended to give an approximate measure of substrate embeddedness only.

Spawning habitat preferences and habitat availability

We first performed an univariate analysis to provide a simple characterization of redd site selection and then a multivariate analysis to identify those combinations of variables that best distinguished redd sites from unused sites. Our use of the latter technique was based on the assumption that habitat selection likely involves simultaneous assessment of many cues (Marthur et al. 1985). Each subsection was evaluated individually for each analysis. Univariate comparisons consisted of comparing values measured at redd sites and randomly selected sites using the Kolmorogov–Smirnov test. The multivariate analysis consisted of discriminant function analysis (DFA), a procedure that creates linear combinations of variables that best distinguish between redd sites and unused sites and then classifies each observation into one of the initial categories based on the multivariate array (Johnson and Wichern 1992). The proportion of randomly selected sites classified as redd sites was used as an estimate of the proportion of the total area in each subsection available for spawning.

We adjusted the prior probabilities (prior information about the proportion of observations in each category) so that the prior probability for the "redd site group" was as small as possible while still classifying all of the redd sites correctly. In some instances, the prior probability had to be high (>0.8) to correctly classify every redd site. In these instances, we relaxed the requirement that every redd site needed to be correctly classified and used the smallest prior probability that resulted in the correct classification of the remaining redd sites. We classified sites using a jackknife cross-validation procedure to assure nonbiased classification (Johnson and Wichern 1992). Finally, we used the Kolmogorov–Smirnov test to determine whether habitat differed between redd sites and those random sites that had been classified as usable.

Effect of habitat availability on redd superimposition

We performed two analyses to determine whether habitat availability could explain the frequency of superimposition. We first performed a multiple regression analysis to determine whether the proportion of individuals superimposing redd sites was correlated

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Table 1. Number of spawning females and number (percent in parentheses) of females superimposing redds in 1993 and 1994 for each species and section.

		1993		1994	
Species	Section	No. of females	No. superimposing (%)	No. of females	No. superimposing (%)
Brown	1	17	3 (18)	31	7 (23)
Brown	2	32	16 (50)	28	11 (39)
Brook	1	1	0 (0)	0	—
Brook	2	18	10 (56)	17	9 (53)

with the amount of available habitat or the number of spawning females in each subsection. We then performed a more detailed analysis to determine whether the observed frequency of superimposition was greater than that expected, given the amount of available habitat and the number of females spawning in each subsection. For each subsection, we estimated the expected frequency of superimposition by simulating random placement of redd sites among available habitat. We assumed that each redd occupied 0.25 m^2 of available habitat (the largest of 25 redds measured in a 1993 pilot study, T.E. Essington, unpublished). For each simulation, we randomly assigned females to available spawning sites and tabulated how many times redds were used multiple times. We performed 1000 simulations for each subsection, species, and year. From these simulations, we calculated the mean, 0.05, and 0.95 percentiles of the number of females superimposing redds. If the observed number of females superimposing redd sites exceeded the 0.95 percentile of the simulations, we concluded that females superimposed redd sites significantly more frequently than expected under the null hypothesis. In subsections in which more than one species dug redds, we determined how many redds of one species were also suitable for use by the other species using DFA and treated them as if they represented a potential redd site throughout the spawning season. For the 1993 data, we did not have habitat measurements on redd sites to perform this analysis, so we assumed that heterospecific redd sites occupied a potential redd site if they were used by heterospecific females.

Experimental test of whether females prefer to superimpose redds

We conducted a simple behavior experiment to directly assess the hypothesis that fish prefer to spawn on existing redd sites. Early in the 1994 spawning season, we selected two experimental sections (2.0 and 5.6 m^2) located in a portion of Valley Creek that was not associated with habitat assessment work. Within each section, two randomly selected locations were designated as "control" locations, while at two additional randomly selected locations, we constructed artificial redds by displacing the substrate with a rubber spatula, approximately simulating the motion of digging females. Both the artificial redds and control sites were then made inaccessible by covering them with large cobble. Later, when females were seen digging redds in these sections, we frightened them away by approaching them from the stream bank and covered their redd site with large cobble substrate while uncovering one control and one artificial redd site. We waited behind a blind until the female returned to the area (usually for about 25 min) and recorded the number and location of all digging bouts for 1 h. We classified sites chosen by females into one of four categories: control site, artificial redd site, undisturbed sites (i.e., sites that we had not previously selected), and previously existing redd sites (i.e., redds that had been constructed by fish before the experiment started). Under the null hypothesis of no preference among categories, the expected number of females selecting sites within any category was equal to the proportion of the total area occupied by each category multiplied by the total number of females observed in each section. We tested whether the observed data conformed to the null hypothesis using the chi-square goodness-of-fit test.

Results

Frequency of redd superimposition

Superimposition occurred frequently during both years for both species, but brook trout superimposed redds more frequently than brown trout (Table 1). Overall, 19 of 36 (53%) female brook trout and 37 of 108 (34%) female brown trout superimposed redds. Within section 2, brook trout superimposed redds more frequently than brown trout during both years, despite the fact that there were fewer spawning brook trout (Table 1). Brown trout in section 2 superimposed redds more frequently than brown trout in section 1 in both years (Table 1). In the 2 years of the study, the 144 females used a total of 80 redds, 73 of which were used by a single species (i.e., there was little interaction between species).

Spawning habitat preferences and habitat availability

Univariate analyses demonstrated that female brown trout tended to select sites with relatively high velocities and greater medium-sized substrate than was present at randomly selected sites. In subsections 1 and 2, brown trout selected spawning sites with faster water velocities and greater proportions of medium substrate than were measured at randomly selected sites, although these differences were not statistically significant (Table 2). In subsection 3, brown trout selected sites with significantly greater water velocity and groundwater upwelling ($P < 0.05$; Table 2). In subsection 4, brown trout selected sites with significantly greater groundwater upwelling ($P < 0.05$; Table 2), but otherwise, redd sites closely resembled randomly selected sites. In contrast, brook trout tended to select sites with groundwater input, but not sites with markedly different water velocities (Table 3). For example, brook trout in subsection 3 selected sites with significantly deeper water and greater groundwater upwelling than were measured at random sites ($P < 0.05$; Table 3). No significant differences were found between randomly selected sites and redd sites in subsection 4, but the same trends were evident.

Multivariate canonical coefficients that distinguished redd sites from unused sites were consistent with the univariate differences in habitat parameters described above (Table 4). For brown trout, coefficients for mean water velocity, bottom velocity, and proportion of medium substrate were consistently positive whereas coefficients for small substrate were consistently negative. The coefficient for depth tended to be positive, except for subsection 3 in which the coefficient was negative, but close to zero. For brook trout,

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Table 2. Mean ± SD of habitat parameters measured at all randomly selected sites and brown trout redd sites.

	Subsection 1		Subsection 2		Subsection 3		Subsection 4	
	Random	Redds	Random	Redds	Random	Redds	Random	Redds
Depth (cm)	21±7	24±5	24±9	34±10	30±9	29±10	44±5	42±7
Mean velocity (cm/s)	52±25	60±10	48±37	57±12	17±6	23±6*	16±5	16±5
Bottom velocity (cm/s)	26±20	26±16	18±17	24±10	6±7	10±5	5±4	4±4
Embeddedness	2.1±0.3	2.0±0	2.2±0.5	2.0±0.0	2.2±0.4	1.9±0.6	2.0±0.4	2.0±0.0
% groundwater	0±0	0±2	10±10	0±0	30±20	50±20*	30±20	40±10*
% small substrate	26±35	17±34	5±36	41±41	44±27	22±26	64±28	62±23
% medium substrate	74±35	81±34	40±37	59±59	46±30	78±26	32±29	38±23
% large substrate	0±0	2±8	2±8	0±0	10±23	0±0	4±15	0±0

*Significant difference between random sites and redd sites for that parameter (Komorogov–Smirnov test, $P < 0.05$).

Table 3. Mean ± SD of habitat parameters measured at 50 randomly selected sites and brook trout redd sites.

	Subsection 3		Subsection 4	
	Random	Redds	Random	Redds
Depth (cm)	30±9	41±5*	44±5	42±3
Mean velocity (cm/s)	17±6	17±4	16±5	17±2
Bottom velocity (cm/s)	6±7	8±4	5±4	4±2
Embeddedness	2.2±0.4	2.0±0.0	2.0±0.4	2.0±0.0
% groundwater	30±20	60±10*	30±20	50±10
% small substrate	44±27	39±19	64±28	60±12
% medium substrate	46±30	61±19	32±29	40±12
% large substrate	10±23	0±0	4±15	0±0

*Significant difference between random sites and redd sites (Kolmorogov–Smirnov test, $P < 0.05$).

groundwater was strongly and positively weighted in both subsections 3 and 4, and medium substrate was consistently positively weighted. All parameters that were significantly different between redd sites and random sites based on univariate comparisons were strongly weighted in the multivariate coefficients.

Jackknife cross-validation demonstrated that relatively large areas of the subsections were indistinguishable from redd sites. Subsection 1 had the greatest percentage of total area classified as usable (75%). Subsection 3 had the least available habitat, with only 2.7 of 33 m^2 (8%) classified as usable for brook trout. In subsections 3 and 4, in which both brook and brown trout spawned, brook trout had less usable area than brown trout. Univariate comparisons of redd sites and the random sites classified as available did not yield any significant differences for any habitat parameter, subsection, or species ($P > 0.1$ for all comparisons).

Effect of habitat availability on redd superimposition

Neither female abundance nor habitat availability was significantly correlated with the proportion of females superimposing redd sites ($P = 0.708$ for female abundance; $P = 0.713$ for habitat availability). In addition, the aggregate of these two independent variables, spawner density (females per square metre of available habitat), was also not correlated with superimposition ($P = 0.46$; Fig. 1). Significance was not improved by evaluating each species separately, and for brook trout the slope was negative (less superimposition at higher spawner densities). Thus, the regression analysis provided no evidence to suggest that redd superimposition might be determined by habitat limitation.

For every subsection and species, the observed frequency of superimposition exceeded the frequency expected if females randomly dispersed over available habitat. For brown trout, the difference between observed and expected superimposition was statistically significant in six of eight instances ($P < 0.05$; Fig. 2). For the two other subsections, the observed frequency was equal to the 0.95 percentile of expected frequencies (Fig. 2). For brook trout, the observed frequency of superimposition was significantly greater than expected in subsection 4 in 1994, and the frequency of superimposition in subsection 3 in 1993 equaled the 0.95 percentile of expected frequencies (Fig. 3).

Behavioral preference for existing redd sites

Female brook trout were observed spawning in the experimental sections on eight occasions, and five females returned to select a new site after being disturbed. Three of these females selected an existing redd site on which to resume spawning, one female selected an artificial redd site, one female selected an unused site, and no females selected a control site. The number of females using existing redds or artificial redds was significantly greater than that expected if females randomly distributed ($\chi^2_3 = 10.89$, $P = 0.01$).

Discussion

The frequency with which females superimposed redds in Valley Creek was striking: in many instances, more than one half of all females had their redds superimposed. These high frequencies are not unique to Valley Creek, as Curry and Noakes (1995) found that between 39 and 60% of brook trout redds in several Ontario streams were superimposed by conspecifics. Further, Beard and Carline (1991) reported that between 50 and 95% of female brown trout superimposed redds in a Pennsylvania stream. In these cases, high frequency of superimposition was attributed to either high spawner densities (Curry and Noakes 1995) or low availability of quality spawning habitat (Beard and Carline 1991). However, neither of these explanations holds for brook and brown trout in Valley Creek. For example, Curry and Noakes (1995) reported redd densities ranging from 2 to 13 redd sites/m^2, yet within our subsections, redd densities never exceeded 0.3 redd site/m^2. Even after correcting for variable habitat quality within subsections, redd densities averaged only 0.94/m^2 available habitat. The difference in

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Table 4. Standardized canonical coefficients that best discriminated between redd sites and randomly selected sites within each subsection (1–4) and estimated total available habitat in each subsection.

	Brown trout				Brook trout	
	1	2	3	4	3	4
Depth	0.37	0.78	–0.08	0.20	0.57	–0.10
Mean velocity	0.35	0.18	0.53	0.06	0.00	0.18
Bottom velocity	–0.03	0.25	0.54	0.22	0.16	0.02
Embeddedness	–0.45	–0.29	–0.47	–0.05	–0.35	–0.04
Groundwater	–0.08	–0.46	0.48	0.67	0.80	0.69
Small substrate	–0.34	–0.35	–0.47	–0.09	–0.10	–0.12
Medium substrate	0.28	–0.38	0.61	0.22	0.29	0.23
Area (m^2) available (% of total)	31.5 (75)	10.2 (60)	7.9 (24)	37.6 (66)	2.6 (8)	29.6 (52)

Fig. 1. Relationship between spawner density and the frequency of redd superimposition observed in subsections for brown trout (solid circles) and brook trout (open circles). Available habitat was estimated by performing DFA on redd sites and randomly selected sites (see text for details).

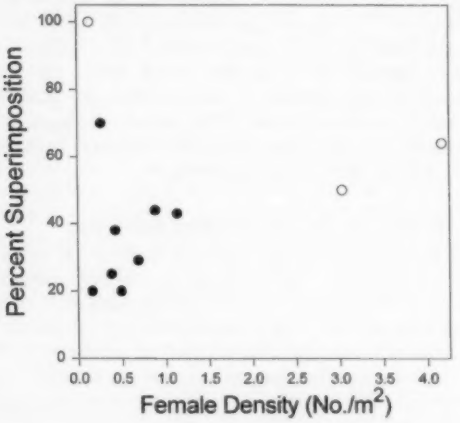
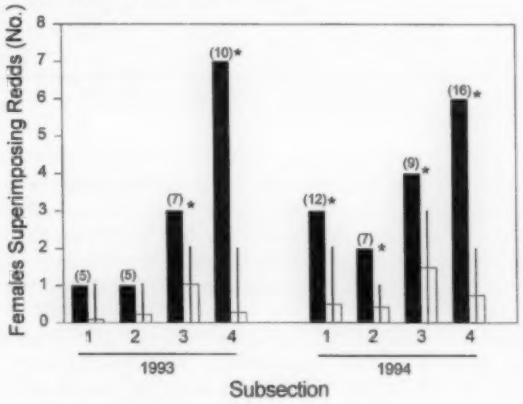


Fig. 2. Observed frequency of brown trout redd superimposition (solid bars) and mean expected frequency (open bars) if females randomly dispersed over available habitat. Lines denote 0.05 and 0.95 percentiles of the expected frequency of superimposition, calculated by performing 1000 simulations of random redd site selection within available habitat. Numbers above bars indicate the total number of females spawning in each subsection. Asterisks denote significant difference between expected and observed superimposition ($P < 0.05$).



redd density is even more striking when one considers that our subsections had the highest densities of spawning fish observed in Valley Creek. Thus, redd superimposition can be an important factor affecting reproductive success even at low densities.

Our analysis of habitat availability provided no evidence that redd superimposition was linked to habitat limitation. Redd superimposition was not correlated with either habitat availability or spawner density, and the frequency of superimposition consistently exceeded that expected if females randomly dispersed over available habitat. For brown trout, this difference was significant in six of eight instances, and for brook trout, this difference was significant in one of three instances. Clearly, females did not randomly disperse within these subsections, but instead, they exhibited marked preferences for a few redd sites. These results strongly suggest that redd site selection is dictated by additional factors other than the physical habitat preferences reported within the literature (e.g., Shirvell and Dungey 1983; Witzel and MacCrimmon 1983; Curry and Noakes 1995).

We argue that the most parsimonious explanation for redd superimposition is that the presence of an existing redd makes a site more attractive for spawning than it would be otherwise. Direct support for this argument comes from our behavior experiment, which indicated that females recognize redd sites and choose to spawn on them when denied access to their own redd. Indirect support for this hypothesis comes from considering alternative hypotheses. One alternative hypothesis is that redd sites were located on the most preferred locations, so that although available habitat was abundant, females sought out the highest quality locations which were limiting and whose presence we were unable to analyze. However, this explanation seems unlikely. Our analytical approach (DFA) was designed to identify the proportion of total habitat that was indistinguishable from redd sites, and sites deemed available by DFA were statistically indistinguishable from redd sites. Further, the habitat preferences reported here are consistent with those reported elsewhere for these two species (Hansen 1975; Shirvell and Dungey 1983; Witzel and MacCrimmon 1983; Curry and Noakes 1995; Sorensen et al. 1995). Moreover, in several instances,

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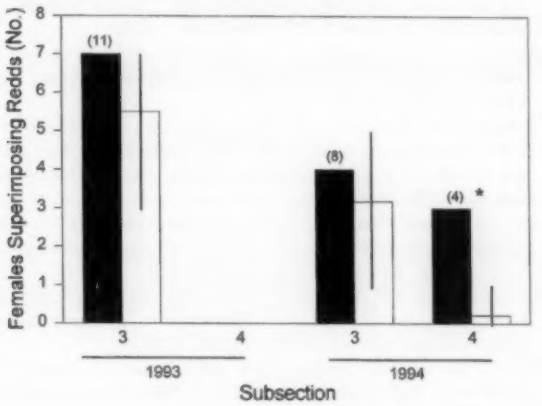
Fig. 3. Observed frequency of brook trout redd superimposition (solid bars) and mean expected frequency (open bars) if females randomly dispersed over available habitat. Lines, asterisks, and numbers above bars are as defined in Fig. 2.



there were no clear differences between redd sites and randomly selected sites. A notable example is brook trout in subsection 4 in 1994, in which every female spawned on the same location, yet this site was not clearly differentiated from much of the available habitat. Further, the fact that this site was initially used by a female brown trout strongly suggests that this site was made more attractive because a redd site already existed there. A second hypothesis is that redd site selection reflects a precise preference for natal sites. However, this hypothesis was ruled out in Valley Creek by an examination of 5 years of records from this location (Sorensen et al. 1995), which demonstrated that although similar locations are used each year, only about 25% of redd sites are used in multiple years.

A third possible explanation of our results is that physical attributes not measured in this study were important in habitat selection. This explanation also seems unlikely. We assessed all habitat parameters previously shown to be important in redd site selection (Shirvell and Dungey 1983; Witzel and MacCrimmon 1983; Curry and Noakes 1995; Sorensen et al. 1995). Also, as an independent check of the possibility that our findings were due to incorrect estimates of habitat availability, we calculated the amount of habitat required for the observed frequency of superimposition to equal the expected frequency. For brown trout, our estimates of habitat availability would had to have been between 2.8 and 16 times too large for habitat alone to explain redd superimposition. For brook trout, our estimates would had to have been 15 times too large to explain superimposition in subsection 4. Again, the most parsimonious explanation is not that our habitat estimates were off by an order of magnitude, but rather that females prefer to spawn on existing redd sites.

It is important to note the scale at which we assessed habitat selection and the nature of the inferences that can be made with this scale of assessment. We assessed redd site selection in small patches containing homogeneous, high-quality spawning habitat. Thus, our analysis does not address the possibility that habitat may be important in determining how female trout choose general regions of streams. In fact, we believe that physical habitat is very important in this regard for two reasons. First, our previous study (Sorensen et al. 1995) showed that not all regions of Valley Creek are used for spawning. Second, the present study identified the same physical factors as being important in localized redd site selection (velocity for brown trout, groundwater for brook trout) as suggested by others on larger scales (Shirvell and Dungey 1983; Witzel and MacCrimmon 1983; Curry and Noakes 1995; Sorensen et al. 1995). Indeed, if spawning habitat had been in very short supply in Valley Creek, we would have expected redd superimposition to be even more frequent. Rather, our study demonstrates that on a microhabitat scale of redd site selection, the presence of existing redd sites may be an important component influencing redd site selection.

It is not surprising that females tend to spawn on existing redd sites. Previously used redd sites may be easier to excavate, thereby reducing energetic costs and mortality associated with digging (Richards and Pickering 1978; Fergusson and Rice 1980). Further, repeated use of a redd site may be effective at removing fine sediment which reduces embryo survival (Hausle and Coble 1976; Chapman 1988). Finally, by destroying eggs of potential competitors, females that superimpose redd sites may enhance the survival of their own offspring. We suggest that future analyses of redd site selection take into account the fact that some areas may be used repeatedly, not because they possess unique habitat characteristics, but simply because the presence of an existing redd makes that site more attractive. More direct tests of this possibility in other streams seem warranted as are evaluations of the effects redd superimposition on salmonine population dynamics.

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References

Beard, T.D., and Carline, R.F. 1991. Influence of spawning and other stream habitat features on spatial variability of wild brown trout. Trans. Am. Fish. Soc. **120**: 711–722.

Chapman, D.W. 1988. Critical review of variables used to define effects of fines in redds of large salmonids. Trans. Am. Fish. Soc. **117**: 1–21.

Curry, R.A., and Noakes, D.L.G. 1995. Groundwater and the selection of spawning sites by brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. **52**: 1733–1740.

Essington, T.E. 1995. Environmental, behavioral, and physiological factors influencing the reproductive success of stream-resident salmonids. M.S. thesis, University of Minnesota, St. Paul, Minn.

Ferguson, H.W., and Rice, D.A. 1980. Post spawning mortalities in brown trout, *Salmo trutta*. J. Fish Dis. **3**: 153–160.

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Fleming, I.A., and Gross, M.R. 1989. Evolution of adult female life history and morphology in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution, **43**: 141–157.

Hansen, E.A. 1975. Some effects of groundwater on brown trout redds. Trans. Am. Fish. Soc. **104**: 100–110.

Hausle, D.A., and Coble, D.W. 1976. Influence of sand in redds on survival and emergence of brook trout (*Salvelinus fontinalus*). Trans. Am. Fish. Soc. **105**: 57–63.

Hayes, J.W. 1987. Competition for spawning space between brown trout (*Salmo trutta*) and rainbow trout (*S. gairdneri*) in a lake inlet tributary, New Zealand. Can. J. Fish. Aquat. Sci. **44**: 40–47.

Johnson, R.A., and Wichern, D.W. 1992. Applied multivariate statistical analysis. Prentice-Hall, Englewood Cliffs, N.J.

Keenleyside, M.H.A., and Dupuis, H.M.C. 1988. Courtship and spawning competition in pink salmon (*Oncorhynchus gorbuscha*). Can. J. Zool. **66**: 262–265.

Ligon, F.K, Dietrich, W.E., and Trush, W.J. 1995. Downstream ecological effects of dams: a geomorphic perspective. BioScience, **45**: 183–192.

Marthur, D., Bason, W.H., Purdy, E.J., and Silver, C.A. 1985. A critique of the instream flow incremental methodology. Can. J. Fish. Aquat. Sci. **42**: 825–831.

McNeil, W.J. 1964. Redd superimposition and egg capacity of pink salmon spawning beds. J. Fish. Res. Board Can. **21**: 1385–1396.

McNeil, W.J. 1966. Randomness in distribution of pink salmon redds. J. Fish. Res. Board Can. **24**: 1629–1634.

Richards, R.H., and Pickering, A.D. 1978. Frequency and distribution patterns of saprologenia infection in wild-and hatchery-reared brown trout, *Salmo trutta* and char, *Salvelinus alpinus*. J. Fish Dis. **1**: 69–82.

Shirvell, C.S., and Dungey, R.G. 1983. Microhabitats chosen by brown trout for feeding and spawning in rivers. Trans. Am. Fish. Soc. **112**: 355–367.

Sorensen, P.W., Cardwell, J.R., Essington, T.E., and Weigel, D.E. 1995. Reproductive interactions between sympatric brook and brown trout in a small Minnesota stream. Can. J. Fish. Aquat. Sci. **52**: 1958–1965.

Tautz, A.F., and Groot, C. 1975. Spawning behavior of chum salmon (*Oncorhynchus keta*) and rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. **32**: 633–642.

van den Berghe, E.P., and Gross, M.R. 1989. Natural selection resulting from female breeding competition in a Pacific salmon (*Oncorhynchus kisutch*). Evolution, **43**: 125–140.

Witzel, L.D., and MacCrimmon, H.R. 1983. Redd site selection by brook trout and brown trout in southwestern Ontario streams. Trans. Am. Fish. Soc. **112**: 760–771.

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Metal accumulation in American wild celery (*Vallisneria americana* Michx.) in the St. Lawrence River: effects of water depth and exposure to current

Christiane Hudon

Abstract: The sensitivity of *Vallisneria americana* Michx. as an indicator species of metal concentrations was assessed through the examination of its spatial (between- and within-site) and temporal (short- and long-term) variability. Eight macrophyte beds located in the St. Lawrence and Ottawa rivers were selected to contrast metal concentrations found in plants exposed to different types of waters, upstream and downstream of their confluence, in the Greater Montreal urban area. Comparisons among sites revealed higher metal concentrations, both in water and in plant tissues, at sites exposed to Ottawa River "brown" waters than at sites exposed to St. Lawrence River "green" waters. Within each site, samples represented a broad range in terms of water depths, exposure to current, incident light intensity, and total plant biomass. At all sites, metal concentrations in plant tissues were lowest in the shallow water found in sheltered, dense beds of submerged aquatic vegetation and increased in deep, open-water areas beyond the limits of dense vegetation. This persistent gradient may result from local differences in plant growth rates, exposure to currents, and (or) metal bioavailability. The use of a sampling strategy designed to control for within-site (depth-balanced) variability makes it possible to measure spatial and (or) temporal differences on the order of 20% whereas unbalanced sampling designs may lead to erroneous conclusions. Significant reductions in metal concentrations in plant tissue were observed both between 1994 and 1996 (Fe, Mn, Pb, Zn, N) and between 1976 and 1996 (Cd, Cr, Pb, Zn, N). Particular care must be given to sampling design if *V. americana* is to be used as a biological indicator of further long-term reductions in metal concentrations.

Résumé : La sensibilité de *Vallisneria americana* Michx. comme espèce indicatrice des concentrations de métaux a été évaluée par examen de sa variabilité spatiale (tant entre différents sites qu'à l'intérieur de chaque site) et temporelle (à court et à long terme). Afin d'illustrer la variabilité des concentrations de métaux dans les tissus de plantes exposées à divers types d'eau, on a choisi huit peuplements de macrophytes dans le Saint-Laurent et l'Outaouais, en amont et en aval du point de confluence de ces deux cours d'eau dans la région métropolitaine de Montréal. Des comparaisons entre les sites ont révélé que les concentrations de métaux, tant dans l'eau que dans les tissus des plantes, étaient plus élevées dans les sites exposés aux eaux « brunes » de l'Outaouais que dans les sites exposés aux eaux « vertes » du Saint-Laurent. À l'intérieur de chaque site, on a prélevé des échantillons représentant la diversité des conditions en ce qui a trait à la profondeur de l'eau, l'exposition au courant, l'intensité de la lumière incidente et la biomasse végétale totale. Dans tous les sites, les concentrations de métaux dans les tissus des plantes étaient plus faibles dans les zones peu profondes abritant des peuplements denses de plantes aquatiques submergées, et plus élevées dans les eaux libres et plus profondes se trouvant à l'extérieur des peuplements denses. Ce gradient pourrait être imputable à des différences locales dans le taux de croissance de la végétation, l'exposition aux courants et (ou) la biodisponibilité des métaux. Le recours à une stratégie d'échantillonnage offrant la possibilité de contrôler la variabilité à l'intérieur des sites (équilibrée en fonction de la profondeur) permet de mesurer des différences spatiales et (ou) temporelles de l'ordre de 20%, tandis que l'utilisation d'un plan d'échantillonnage non équilibré peut mener à des conclusions erronées. Des réductions significatives des concentrations de métaux dans les tissus des plantes ont été observées entre 1994 et 1996 (Fe, Mn, Pb, Zn, N) et entre 1996 et 1997 (Cd, Cr, Pb, Zn, N). Il importe d'accorder une attention particulière au plan d'échantillonnage si l'on prévoit utiliser *V. americana* comme indicateur biologique en vue de déceler des réductions à plus long terme des concentrations de métaux.

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Introduction

Species of submerged aquatic vegetation (SAV) are often used as indicator organisms to monitor metal contamination in aquatic environments (Ray and White 1979; Greger and Kautsky 1992). Although SAV is effective in characterizing

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differences in metal concentrations across several orders of magnitude in a wide variety of water bodies (Jackson et al. 1991), correlations between metal concentrations in SAV and those in water and (or) sediments are more difficult to establish (Campbell et al. 1985; review by Outridge and Noller 1991), especially across the narrower range normally encountered within a single water body. In addition, an effective indicator should be sensitive enough to allow the early detection of spatial and temporal changes in metal concentrations in the ecosystem. The sensitivity of indicators can be enhanced by optimizing sampling strategies to eliminate unwanted sources of variability in metal concentrations, especially when variations within and among sites are equal to, or greater than, the temporal changes to be measured.

Metal concentrations in SAV have been shown to respond to the combined effects of sediment geochemistry, water chemistry, plant physiology, and genotypic differences (Outridge and Noller 1991). Monitoring programs should control for plant species (Ray and White 1976; Mudroch and Capobianco 1979; Sprenger and McIntosh 1989; St-Cyr and Campbell 1994), time of collection (Dykyjová 1973; Gommes and Muntau 1981), and anatomic structure and plant tissue (Miller et al. 1983). Metal concentrations in SAV can also vary with biological factors such as seasonal metabolism and growth (Mudroch and Capobianco 1979). Slow-growing plants collected in deep or turbid waters, and thus exposed to low light intensity, would accumulate more metals than fast-growing plants exposed to high light intensity. Consequently, monitoring studies controlling for plant species, plant section, and time of collection might still reach erroneous conclusions if differences in growth conditions within and among sites are not considered.

In addition, sampling design must account for the interaction and retroactive feedback occurring between geochemical and biological factors under field conditions. SAV alters the characteristics of surrounding water and sediment (pH, redox potential, concentrations of Fe and other chelating agents) and vice versa, all of which influence the biological availability of metals (Campbell et al. 1985). The presence of SAV has been shown to reduce current speed and favour retention of the fine particles with which metals are associated. Water circulation has also been shown to vary within SAV beds (Smith et al. 1995), thus generating small-scale gradients in sediment grain size, oxygenation level, and organic content and, more importantly, creating potential differences in dissolved metal concentrations and bioavailability. Metal concentrations found in plant tissues thus appear influenced by the combination and interaction of geochemical, biological, and ecological factors that covary with the position and density of plants within a macrophyte bed. Plants in very dense beds would be removed from the effects of currents and sheltered from the inflow of particulate matter. Conversely, plants in less dense beds or on the periphery of beds would be exposed to higher water exchange at slow current speed, thus favouring the sedimentation of fine, contaminated particles.

This study assesses the sensitivity of *Vallisneria americana* Michx. as an indicator species of metal concentrations through the examination of its spatial (between- and within-site) and temporal (short- and long-term) variability. Eight macrophyte beds located in the St. Lawrence and Ottawa rivers were selected to contrast metal concentrations found in plants exposed to different types of waters, upstream and downstream of their confluence, in the Greater Montreal urban area. Variability of metal concentrations within each bed was assessed by collecting plants along transects in sheltered, shallow water and progressing to the limit of plant occurrence in deep, open water. The consequences of different sampling design on the assessment of temporal changes were then examined using short-term (1994–1996) and long-term (1976–1996) data.

Study area

The study area comprises a roughly 100-km-long section of the St. Lawrence (mean annual discharge 7000 m^3/s) and Ottawa (mean annual discharge 1980 m^3/s) rivers, extending about 60 km downstream of their confluence to Verchères (Fig. 1). The upstream area includes fluvial lakes Saint-François (40 km upstream of Montreal) and des Deux Montagnes (20 km upstream of Montreal), which are widenings of the St. Lawrence and Ottawa rivers, respectively. Lake Saint-François waters are clear (≥5 m Secchi), low-P (<7 μg PO_4-P/L), moderately mineralized (>275 μS/cm) green waters outflowing Lake Ontario (Table 1). By contrast, Lake des Deux Montagnes receives the low-clarity (≤2 m Secchi), soft (<100 μS/cm) brown waters draining the Canadian Shield, which are enriched with P (>10 μg PO_4-P/L) as they flow through the farmlands of the Ottawa–Hull region.

The passage of these waters through the Greater Montreal urban area (population 3 million people) greatly increases their suspended matter concentrations due to erosion and human activities (Hudon and Sylvestre 1998). The south side of the Island of Montreal (the shores of Lake Saint-Louis, La Prairie Basin, and Montreal) is heavily urbanized and subject to storm sewer overflows of combined domestic and industrial waste waters in Montreal Harbour. Waters from the Ottawa River and Lake des Deux Montagnes flow in part along the south side of the island of Montreal and Montreal Harbour (Désilets and Langlois 1989). The remainder of Ottawa River discharge flows through the des Prairies and des Mille Îles rivers, which still receive raw domestic and industrial sewage from the city of Laval (northern suburbs of Montreal). The confluence of the St. Lawrence and Ottawa rivers occurs in the fluvial corridor downstream of the Island of Montreal; brown water from the Ottawa River flows along the north shore (left bank) whereas green St. Lawrence River water dominates in the main navigation channel and along the south shore (right bank), with a median mixing zone that varies seasonally.

Spatial variations resulting from water quality characteristics also coincide with differences in total metal concentrations present in the water, depending on site location. Waters of the St. Lawrence River exhibit lower metal and suspended matter concentrations than the Ottawa River (Table 2). Within each river, metal concentrations increase significantly downstream, in part owing to the large increase in suspended particulate matter in both water masses.

Regardless of the differences in water quality and metal concentrations, *V. americana* is the dominant submerged macrophyte species in both rivers, where it is found over a

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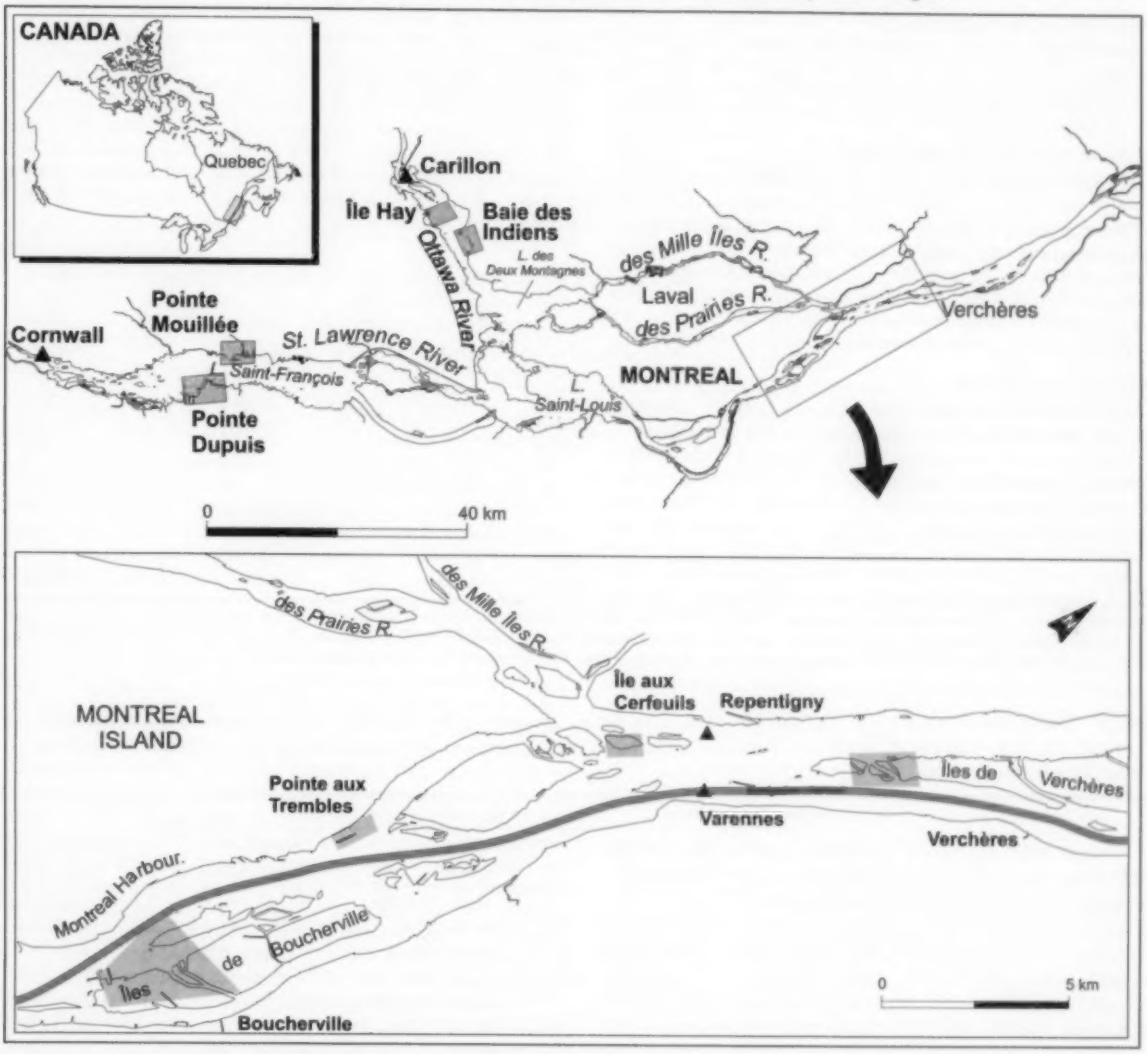
Fig. 1. Location of the sampling sites within the study area. Shaded areas in the upper panel indicate the four sites upstream of the confluence of the Ottawa (Lake des Deux-Montagnes: Baie des Indiens, Île Hay) and St. Lawrence rivers (Lake Saint-François: Pointe Dupuis, Pointe Mouillée). The lower panel shows the four sites downstream of the Greater Montreal area, along the north (Pointe aux Trembles, Île aux Cerfeuils) and south (Boucherville, Verchères) sides of the river. Water sampling stations upstream (Cornwall, Carillon) and downstream (Varennes, Repentigny) of the Greater Montreal area are indicated by solid triangles.



broad range of water depth, light intensity, current speed, and sediment type (St-Cyr and Campbell 1994; Hudon 1997) (Table 1). Since this species produces new above- and below-ground tissues every year from small overwintering structures, it is well suited to monitoring yearly variations in metal concentrations. However, total aboveground biomass of SAV is lower (<200 g dry plant mass/m^2) upstream of the confluence than downstream (<400 g dry plant mass/m^2) for both rivers (Hudon and Lalonde 1998).

Materials and methods

Vallisneria americana was collected from eight submerged aquatic plant beds located upstream and downstream of the Greater Montreal urban area (Fig. 1). Sampling sites were selected to represent the different types of SAV beds occurring in the broad fluvial lakes and the narrower fluvial corridors of the St. Lawrence River. Upstream sites located in lakes Saint-François (two sites) and des Deux Montagnes (two sites) were sampled in 1996 whereas four sites in the fluvial corridor downstream of Montreal were sampled in both 1994 and 1996 (Table 1). Sites located in fluvial lakes sloped gently from the shoreline to the limit of plant growth, which stretched over a distance of about 1 km from shore in these shallow fluvial sections. In the fluvial corridor, two of the sites (Île de Pointe aux Trembles, Île aux Cerfeuils) consisted of narrow bands (about 100 m wide) with dense SAV beds in the shallow part of the littoral zone, followed by a steep slope to the limit of submerged vegetation (Fig. 2). The last two sites (Boucherville, Verchères) were more complex, since they con-

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Table 1. Description of characteristics of *V. americana* sampling sites.

	St. Lawrence River waters				Ottawa River waters			
	Upstream		Downstream		Upstream		Downstream	
	LSF-PD	LSF-PM	BOU	VER	LDM-IH	LDM-BI	PAT	IAC
Water transparency (min., max.)								
Secchi depth (m)	5	5.6	2.5–3	0.5–2	2	1.5	2.3	2.5
k (m^{-1})	0.33	0.28	0.58–0.72	0.62–1.67	1.30	1.34	0.71–0.83	0.73–0.95
% of surface light reaching *V. americana* (I_z/I_0)	17–81	21–80	5–58	1–54	3–46	9–42	4–84	5–72
Chemical characteristics (mean (SD))								
Conductivity (µS/cm)	288 (16)	313 (17)	292 (32)	305 (24)	83 (23)	71 (15)	276 (38)	284 (38)
pH	8.2 (0.2)	8.2 (0.2)	8.2 (0.2)	8.1 (0.1)	7.3 (0.3)	7.3 (0.3)	8.1 (0.2)	8.1 (0.1)
Total organic C (mg/L)	2.4 (0.4)	2.4 (0.5)	2.6 (0.6)	2.2 (0.4)	5.6 (0.6)	5.6 (0.6)	3.0 (0.8)	2.9 (0.6)
Sediment type (% frequency)								
Gravel	7	nd	38	nd	nd	nd	nd	nd
Sand	93	71	nd	21	78	85	19	83
Clay–silt	nd	25	61	79	22	15	81	17
Organic	nd	4	nd	nd	nd	nd	nd	nd
Sampling characteristics								
Morphology of macrophyte bed	Littoral	Littoral	Channel	Channel	Littoral	Littoral	Littoral	Littoral
Depth range of *V. americana* samples (m)	0.6–5.2	0.8–5.5	0.7–4.5	0.75–3.75	0.6–2.8	0.7–1.8	0.25–4	0.35–3.4
Distance of farthest *V. americana* sample from the shore (km)	1.7	1.3	3	1.3	0.7	1	0.10	0.13
No. above-/below-ground tissue samples								
1994	na	na	11/11	9/9	na	na	16/13	17/14
1996	6/0	5/0	10/10	10/10	6/0	5/0	10/10	10/10

Note: Sites: LSF-PD, Lake Saint-François–Pointe Dupuis; LSF-PM, Lake Saint-François–Pointe Mouillée; BOU, Boucherville; VER, Verchères; LDM-IH, Lake des Deux-Montagnes–Île Hay; LDM-BI, Lake des Deux-Montagnes–Baie des Indiens; PAT, Pointe aux Trembles; IAC, Île aux Cerfeuils. Water quality data obtained from NAQUADAT monitoring stations (Rondeau 1993). na, not available; nd, not detected.

Table 2. Comparison of mean metal concentration (SD, n) in the St. Lawrence River and Ottawa River waters sampled upstream (Cornwall, Carillon; Cossa et al. 1998) and downstream (Varennes (site 2), Repentigny (site 8); Hudon and Sylvestre 1998) of the Greater Montreal urban area.

	St. Lawrence River waters		Ottawa River waters		
Metal	Upstream (Cornwall)	Downstream (Varennes)	Upstream (Carillon)	Downstream (Repentigny)	$ANOVA_{station}$ F (p)
Cd (ng/L)	13.6*a* (5.2, 5)	na	21.8*b* (7.1, 28)	na	7.69**
Cu (ng/L)	692.5*a* (77.5, 22)	1075.0*b* (134.1, 14)	1244.9*b* (521.3, 29)	2871.4*c* (2309.0, 14)	44.79***
Fe (µg/L)	27.3*a* (11.9, 30)	163.8*b* (98.4, 14)	484.4*c* (588.9, 26)	787.4*d* (720.5, 14)	134.38***
Mn (µg/L)	2.3*a* (1.2, 30)	6.0*b* (2.6, 14)	20.4*c* (10.5, 29)	25.8*c* (16.6, 14)	122.19***
Ni (ng/L)	686.0*a* (82.8, 19)	na	1014.1*b* (446.8, 24)	na	11.37**
Pb (ng/L)	34.5*a* (12.7, 16)	na	244.9*b* (154.3, 28)	na	48.40***
Zn (ng/L)	574.2*a* (204.8, 13)	1260.7*a* (552.7, 14)	2865.0*a* (2011.8, 26)	4664.3*b* (3251.3, 14)	5.41**
Suspended particulate matter (mg/L)	0.8*a* (0.4, 30)	4.9*b* (2.7, 14)	8.2*b* (11.4, 32)	17.3*c* (18.4, 14)	73.38***

Note: Location of water sampling stations is indicated in Fig. 1. Upstream values correspond to the sum of concentrations measured separately in dissolved and particulate fractions from large-volume sampling. Downstream values represent total extractible metal concentration from a 1-L water sample. Values obtained from samples taken at regular interval over the course of 1 year (May 1995 – May 1996) are compared for the four stations. na, data not available. Values followed by the same letter did not differ significantly (ANOVA and Duncan test of comparisons of means on $\log_{10}$-transformed data). Probability (p) associated with ANOVA F values: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

sisted of large SAV beds located in 1- to 3 km-long channels between islands. Dense SAV covered most of these channels, except for the deeper areas where water circulates slowly (<0.3 m/s).

Sampling

Littoral sites were sampled at the shallow, nearshore area of the SAV bed up to the limit of plant occurrence in deep water. Channel sites were sampled longitudinally in the direction of the current, from their upstream edge towards the inside (Boucherville), through the downstream edge of vegetation to its deepwater limit (Verchères). In Boucherville, pairs of samples were taken from within the channel to compare contamination levels in plants exposed to a slow current versus those located in the adjacent dense SAV bed. Depending on the size, slope, and morphology (littoral

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or channel) of the site, between 5 and 17 samples were taken per site on each occasion.

Vallisneria americana was collected by scuba divers within a 5-m radius at roughly equal intervals (depending on plant occurrence) along a line extending from the shore to the limit of plant distribution in deep water. Whole plants (between 10 and 25 individual plants, depending on their size) were gently uprooted after loosening surrounding sediments by hand and pooled to obtain sufficient plant material for analyses. Plant samples were first washed of sediment and loose periphyton before being placed into plastic bags and kept in coolers until transport to the laboratory for further processing. Total biomass of above- and below-ground SAV was sampled simultaneously using 25 × 25 cm quadrats and converted to grams dry plant mass per square metre (Hudon 1997; Hudon and Lalonde 1998).

Field measurements

At each site, measurements of conductivity (Hydrolab Surveyor III), water transparency (20-cm Secchi disk), and underwater light penetration (Licor aerial probe SA-LI188B and underwater probe SA-LI192SA) were made adjacent to dense SAV beds. Light extinction coefficient (k) was calculated as the slope of the regression of light intensity at increasing depth ($\ln I_z$) against depth (z). Fraction of surface light intensity (I_z/I_0) at collection depth (z) was calculated as $I_z/I_0 = e^{-kz}$, expressed as a percentage. Sediment type within SAV beds was assessed at each site by the qualitative examination of every 5 m along a transect line extending from dry ground to the edge of dense SAV beds (about 1 m depth). Current speed was measured at each site outside of dense SAV using either an electromagnetic (Montedoro Whitney PVM-2A) or a mechanized (Price 622 AA) current meter.

Laboratory analyses

Above- (leaves only) and below-ground tissues of *V. americana* were separated by hand and remaining traces of periphyton, loose detritus, and sediment carefully washed off with tap water. Samples were subsequently washed in deionized water, dried to a constant mass (at 105°C), and ground manually. To minimize contamination, all labware was soaked in HCl and rinsed in deionized water prior to use. Total N was analysed using a carbon–hydrogen–nitrogen analyzer (Environment Canada 1979*a*). Acid-digested samples were analyzed for total P using a colorimetric method in 1994 (Environment Canada 1979*b*) or inductively coupled plasma mass spectrometry (ICP-MS) in 1996 (Environment Canada 1994*a*). Metals were measured after acid digestion (H_2SO_4, HNO_3, H_2O_2) using flameless graphite furnace atomic absorption spectrophotometry (AA-GF) (Pb in 1994), ICP-MS (Cd, Ni, Pb in 1996), or AA (all other metals in 1994 and 1996) (Environment Canada 1994*a*, 1994*b*). Certified reference materials from the U.S. National Institute of Standards and Technology (*Pinus* needles SRM 1575 and *Citrus* leaves SRM 1572) were digested during each analytical run; measured metal concentrations were consistently within the certified ranges for each element. Recovery of metal in spiked samples was always >85%. Repeated analysis, in 1994 and 1996, of a single sample of *V. americana* material originating from the combination of all leftover dry plant material in 1994, as an additional reference, yielded nearly identical results for all metals. Analytical error, expressed as the coefficient of variation obtained from analysis of unidentified duplicate samples, was ≤7% in all but three analyses out of 84 (7 metals × 12 pairs of replicates) (Hudon and Lalonde 1998).

Data analyses

Comparisons of metal concentrations among sites and years were carried out using one-way ANOVAs (SAS PROC GLM) on $\log_{10}$-transformed data or paired t-tests. *Vallisneria americana* samples were individually characterized with respect to collection depth (metres) and relative position from the edge of the dense SAV bed, as defined by the limit of thick vegetation reaching and floating on the surface. To allow for the common treatment of all sites and compensate for the large differences in SAV bed size, the position of samples with respect to the edge of the dense SAV bed (distance value in Table 1) was transformed into ranks (–12 to +12). Characteristics of site morphology (littoral, channel) and current speed were integrated to create three classes of exposure to water movements: sheltered (class 0: no current), moderately exposed (class 1: current <0.3 m/s), and exposed sites (class 2: current >0.3 m/s).

Metal concentrations were normalized ((individual values – mean)/standard deviation) for each site and year to facilitate the comparison of small-scale concentration gradients with depth and distance from shore among sites differing in absolute metal concentrations. Environmental variables were correlated with normalized metal concentrations, thus allowing us to use data from all sites and both collection years. Appropriate correlation indices (SAS PROC CORR) were used for categorical (Spearman rank) and quantitative (Pearson r parametric index) environmental variables.

Metal concentrations recorded in 1976 (Béland and Demers 1977, n = 70) were compared with values from 1990 (St-Cyr and Campbell 1994, n = 25) and from 1994 and 1996 (this study) using ANOVA (SAS PROC GLM). Whereas recent metal concentrations were derived from aboveground tissues of *V. americana*, the 1976 study used three species (*V. americana*, *Ceratophyllum demersum*, and *Myriophyllum* sp.), including some below-ground tissues of the last one. However, the comparison was nevertheless deemed valid, since between-species comparisons carried out in the St. Lawrence River (St-Cyr and Campbell 1994; Hudon and Lalonde 1998) showed that *V. americana* concentrates metals more efficiently than do other species of SAV. In addition, below-ground tissues represent a negligible fraction of total *Myriophyllum* sp. biomass (Hudon and Lalonde 1998). Therefore, the exclusive use of *V. americana* in recent studies likely yielded a conservative estimate of the differences over time.

Results

Metal concentration in plant tissues at eight sites

Sites differed markedly in terms of water transparency and chemical characteristics, which are primarily a reflection of the origin of the water masses found at each site. The high water transparency and conductivity observed at upstream St. Lawrence River sites (Lake Saint-François) readily distinguished them from Ottawa River sites (Lake des Deux Montagnes) (Table 1). Sites located downstream of the Greater Montreal area exhibited intermediate transparency and total organic C concentrations resulting from the mixing of the two upstream water masses. Downstream sites also exhibited higher variability in conductivity and other chemical characteristics than upstream sites, resulting from seasonal differences in discharge and mixing conditions. The Boucherville and Verchères sites, located closer to the south shore of the river, were more strongly influenced by green waters whereas the influence of brown waters was more evident at sites at Île aux Cerfeuils and Pointe aux Trembles (Table 1).

Significant differences in metal concentration in aboveground tissue of *V. americana* were detected among sites in 1996 (Table 3). Similarly to the pattern observed with metal concentrations in water (Table 2), sites in St. Lawrence

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Table 3. Comparison of mean metal concentration (SD) in aboveground tissues of *V. americana* sampled in 1996 at eight sites in the St. Lawrence and Ottawa rivers, upstream and downstream of their confluence in the Greater Montreal urban area.

Metal (mg/kg dry plant mass)	St. Lawrence River waters				Ottawa River waters				$ANOVA_{site}$ F (p)
	Upstream		Downstream		Upstream		Downstream		
	LSF-PD (n = 6)	LSF-PM (n = 5)	BOU (n = 10)	VER (n = 10)	LDM-IH (n = 6)	LDM-BI (n = 5)	PAT (n = 10)	IAC (n = 10)	
Cd	0.66*a* (0.50)	0.62*a* (0.29)	0.96*ab* (0.61)	0.80*ab* (0.51)	3.50*d* (1.96)	1.28*abc* (0.67)	2.08*cd* (1.17)	1.70*bc* (1.17)	5.64***
Cr	1.2*a* (0.4)	2.2*ab* (0.8)	4.6*b* (3.1)	3.3*b* (2.2)	4.5*b* (2.4)	4.5*b* (1.7)	3.3*b* (2.5)	3.5*b* (1.2)	2.55*
Cu	9.3*a* (5.2)	12.3*b* (2.0)	13.6*b* (4.7)	12.3*b* (4.2)	20.5*cd* (19.6)	12.8*b* (2.1)	28.9*d* (7.5)	18.9*c* (5.2)	12.24***
Fe	228*a* (56)	159*a* (53)	1064*bc* (452)	922*c* (485)	1977*c* (1119)	881*b* (965)	1270*bc* (853)	1354*bc* (618)	17.71***
Mn	958*a* (255)	783*a* (214)	1398*ab* (801)	1785*bc* (849)	3147*de* (1093)	3192*e* (1749)	2310*cde* (698)	1723*bcd* (664)	6.84***
Ni	6.91*a* (4.25)	9.34*ab* (4.52)	11.27*ab* (6.30)	9.57*ab* (3.42)	18.87*c* (6.92)	13.33*bc* (4.59)	16.97*bc* (7.40)	11.02*abc* (4.47)	3.11**
Pb	1.31*ab* (1.13)	0.85*a* (0.25)	1.75*bcd* (0.68)	1.17*abc* (0.46)	1.01*a* (0.66)	0.98*ab* (0.14)	2.68*d* (0.92)	1.98*cd* (0.86)	5.83***
Zn	63.5*abc* (7.2)	70.9*abc* (12.0)	84.2*bc* (37.7)	56.7*ab* (15.6)	155.0*d* (48.2)	54.4*a* (29.0)	156.6*d* (63.6)	91.3*c* (31.5)	10.41***
N (%)	2.68*a* (0.62)	3.30*b* (0.55)	3.24*b* (0.27)	3.72*b* (0.53)	3.73*b* (0.47)	3.25*b* (0.97)	3.49*b* (0.40)	3.07*ab* (0.19)	3.54**
P (%)	0.22*a* (0.10)	0.33*bc* (0.10)	0.33*bc* (0.09)	0.48*de* (0.05)	0.35*bc* (0.07)	0.25*ab* (0.06)	0.58*e* (0.23)	0.41*cd* (0.10)	9.88***

Note: Full site names are given in Table 1. Values followed by the same letter did not differ significantly (ANOVA and Duncan test of comparisons of means on $\log_{10}$-transformed data). Probability (p) associated with ANOVA F values: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

River green waters supported *V. americana* with consistently lower metal concentrations in their tissue than sites influenced by Ottawa River brown waters (Table 3). The lowest concentrations of Cd, Cu, Fe, Mn, Ni, Pb, and Zn in plant tissues were found at the two sites in Lake Saint-François and in Verchères, located upstream and downstream, respectively, of Montreal, in the green St. Lawrence River waters. In contrast, the highest concentrations of Cd, Cu, Fe, Mn, Ni, and Zn in aboveground tissue of *V. americana* were recorded at two sites under the influence of Ottawa River water, at different ends of the study area: Île Hay (Lake des Deux Montagnes) and Pointe aux Trembles (Table 3). Within water mass, differences between upstream and downstream concentrations of metal in aboveground tissue of *V. americana* were not as clearly defined as those found for metal concentrations in the water. Plants harvested from St. Lawrence River sites showed a consistent downstream increase in metal concentrations (especially Cd, Cr, Fe, and Mn) whereas no systematic downstream increase could be detected for plants growing in Ottawa River waters (Table 3).

Within-site variations in metal concentration in plant tissues

The small-scale variations in metal concentrations in *V. americana* tissues were then examined at the eight sites in order to relate these differences to local environmental factors. *Vallisneria americana* samples were collected from a wide range of depths (0.25–5.5 m), distance from shore (0.1–1.7 km), and light intensity (Table 1). Normalized residual tissue concentrations were used for this exercise, since metal concentrations in tissue were shown to differ significantly from site to site (see previous section, Table 3).

As hypothesized, consistent small-scale spatial gradients were observed: at all sites, metal concentrations in aboveground tissues of *V. americana* were relatively lower in shallow water and increased substantially in deep water (Fig. 2). Although Fig. 2 illustrates only the case for Cd, significant positive correlations with depth were found for all metals (Table 4). The effect of water depth was apparent regardless of the slope, depth range, or sediment type, which differed markedly among sites (Table 1). Fluvial lake bottoms dropped progressively along a shallow slope to a deeper (5–15 m) main channel (Figs. 2A–2D) whereas the bottom of the fluvial corridor formed a shallow (<2 m) shelf around islands and shoals, dropping sharply along a steep slope to deeper waters (Figs. 2E and 2F). Sediment type at each site presented no obvious gradient with depth and showed a predominance of sand and fine (loam and clay) fractions at all sites, with the occasional presence of gravel (Lake Saint-François–Pointe Dupuis, Boucherville) or organic sediments (Lake Saint-François–Pointe Mouillée) (Table 1).

Sharp changes in residual tissue concentration also appeared to stem from the transition from a dense SAV bed to the open water (Fig. 2). Further examination of residual Cd concentration in *V. americana* tissues revealed that *V. americana* samples taken from dense SAV beds exhibited lower metal concentrations in their tissue than in adjacent open-water areas (Figs. 2E, 2F, and 2H). This pattern was observed regardless of the differences in total SAV above-

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ground biomass and vertical distribution of SAV among sites, most likely resulting from local differences in water clarity and extinction coefficients (Table 1). For example, depending on the site, the depth range of *V. americana* corresponded to 1–84% of incident surface light intensity at sampling depth.

Lastly, the presence of a water circulation channel in SAV beds appeared to influence residual metal concentration in aboveground tissues of *V. americana*. *Vallisneria americana* samples collected in dense, sheltered SAV in the 3-km-long Boucherville channel exhibited lower residual metal concentrations than did samples taken in the adjacent channel (Fig. 2G). Similarly, plant tissues sampled within the sheltered Verchères channel showed relatively lower metal concentrations than at either of its open-water extremities (Fig. 2H).

In summary, low residual metal concentrations in aboveground tissues of *V. americana* were observed in shallow-water, densely vegetated, sheltered areas. This pattern was consistent at all sites, whether the depth gradient was progressive (lake sites) or steep (fluvial corridor sites), regardless of maximum depth, distance from shore, or presence of dense SAV. However, since depth gradient and presence of dense SAV were both factors in this regard, it was not possible to distinguish which variable had the strongest effect on residual metal concentration.

The correlations between depth, exposure to current, light intensity, and normalized residual concentrations in *V. americana* tissues were examined separately for each metal, in both above- and below-ground tissues (Table 4). Figure 3 presents the example of plots for residual Cd concentrations in aboveground tissues of *V. americana* and each environmental variable; correlation coefficients for each metal are presented in Table 4. Metals found in aboveground tissues were generally correlated with environmental variables whereas below-ground tissues exhibited few (only Cd and Pb) significant correlations (Table 4); the description of results will therefore focus on aboveground tissues.

As was the case for most elements (Table 4), the plot of normalized Cd concentrations against depth (Fig. 3A) showed a significant positive correlation with a broad scattering of points. Relative position of samples with respect to the edge of dense SAV (Fig. 3B) was negatively correlated with normalized metal concentrations. Samples located within dense SAV were all characterized by below-average element concentrations. However, samples located at the edge of dense vegetation (position = 0) or in open water (position >0) exhibited a broad scattering of residual Cd concentrations (Fig. 3C). Identification of sites and of water mass revealed uniform residuals with depth and position (Figs. 3A and 3B), although this was not always the case with light intensity (Fig. 3C).

Strong negative relationships were observed between the proportion of surface light intensity at sampling depth and normalized metal concentrations (Table 4; Fig. 3C). Samples of *V. americana* growing under low light intensity showed relatively higher metal concentrations in their tissue than did those exposed to high light intensity, thereby suggesting a negative relationship with plant growth rate. Further, for a given light intensity, samples collected in Lake des Deux Montagnes had lower Cd concentrations than those from Lake Saint-François (Fig. 3C); this was also true of Mn, Ni, and Zn (Table 4). Downstream sites exhibited weak or no relationship between light intensity and normalized metal concentrations.

Fig. 2. Variations of residual Cd concentrations ((individual values (x) – mean)/SD) in aboveground tissues of *V. americana* sampled at increasing distance from the shoreline at each of the eight sites in 1996. For each site, the depth profile (solid line) is indicated on the right y-axis. To allow comparison between sites and sampling seasons, distance (x-axis) was transformed into ranks (–12 to +12), where the 0 position (dotted vertical line) coincides with the edge of dense SAV. Negative x-axis values represent samples taken a increasing distance towards the inside of the dense SAV bed (shoreward); positive x-axis values indicate samples taken in open waters away from dense plants. Fluvial lakes (Figs. 2A–2D) were devoid of dense SAV beds. In the Boucherville channel, pairs of adjacent samples were taken in the deep central channel (open circles, dotted depth profile) and in shallow areas of dense SAV growth (solid circles, solid depth profile). In the Verchères channel, samples were taken from the upstream area, across the shallow, dense SAV bed to the downstream end of the channel. Arrows indicate the direction of current in Boucherville and Verchères channels.

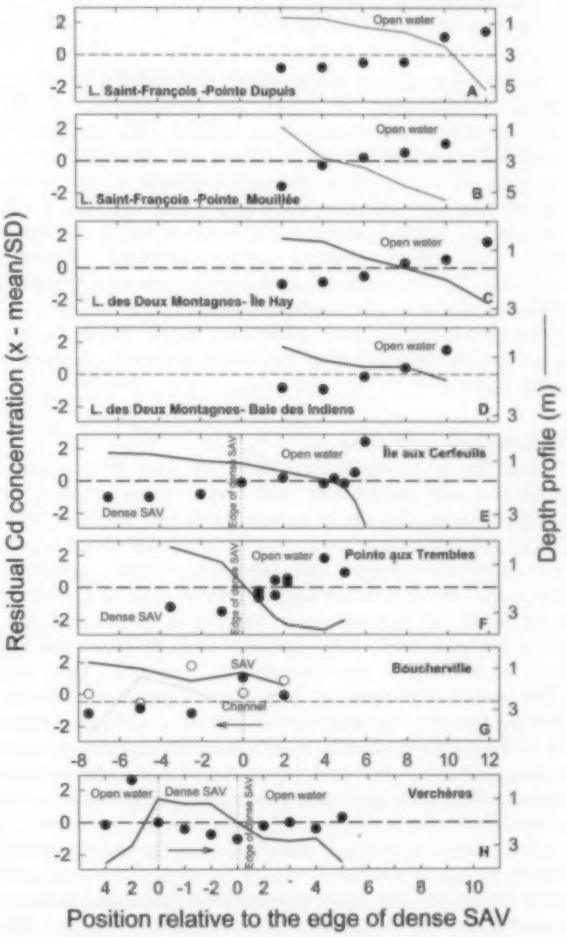

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Table 4. Correlation coefficients between characteristics of sampling sites and normalized metal concentrations in the aboveground (A) and belowground (B) parts of *V. americana* in the St. Lawrence and Ottawa rivers (1994 and 1996).

Metal	Depth ($A_{sign.}/B_{sign.}$)[a]	Relative position ($A_{sign.}/B_{sign.}$)[b]	Fraction of surface light I_z/I_0 ($A_{sign.}/B_{sign.}$)[a]	Water mass interaction (A/B)	Exposure to current ($A_{sign.}/B_{sign.}$)[b]	Total aboveground plant biomass ($A_{sign.}/B_{sign.}$)[a]	Total belowground plant biomass ($A_{sign.}/B_{sign.}$)[a]
Cd	0.55***/0.24*	0.52***/0.22*	–0.54***/ns	Y/N	0.65***/0.51***	ns/ns	–0.25*/ns
Cr	0.29**/ns	0.36***/ns	–0.31***/ns	N/N	0.41***/ns	–0.29*/ns	ns/ns
Cu	0.39**/ns	0.33**/ns	–0.32*/ns	N/N	0.40***/ns	ns/na	ns/na
Fe	ns/ns	0.27**/ns	–0.23*/ns	N/N	0.29**/ns	–0.26*/ns	ns/ns
Mn	0.24**/ns	0.24**/ns	–0.32***/ns	Y/N	ns/ns	ns/0.44**	ns/ns
Ni	0.58***/ns	0.50***/ns	–0.63***/ns	Y/N	0.63***/ns	ns/ns	–0.33**/ns
Pb	ns/–0.37***	ns/–0.28*	ns/–0.46***	N/N	0.24*/ns	–0.36**/ns	–0.25*/ns
Zn	0.47***/ns	0.49***/ns	–0.45***/ns	Y/N	0.64***/ns	ns/ns	–0.29*/ns
N	ns/ns	ns/ns	ns/ns	N/N	ns/ns	–0.33**/na	–0.26*/na
P	0.32**/ns	0.28**/ns	–0.33***/ns	N/N	ns/ns	ns/na	–0.37**/na

Note: The presence of a significant interaction with water mass (Yes (Y), No (N)) indicates the occurrence of different relationships for Lakes Saint-François and des Deux Montagnes. na, not available. Number of samples in correlations vary between 40 and 115. Significance: ns, not significant; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. na, data not available.

[a]Pearson correlation coefficient.

[b]Spearman rank correlation coefficient.

Exposure to current was positively correlated with relative metal concentrations (Table 4). The identification of SAV samples taken from environments ranging from sheltered to current exposed and plotted on the same axes (Fig. 3D) illustrates the simultaneous, interactive effects of exposure to current and light intensity. For a given light intensity, plants exposed to currents exhibit higher relative metal concentrations than plants growing under sheltered conditions (Fig. 3D). Lastly, weak negative correlations were observed between total aboveground/below-ground plant biomass and metal concentrations in *V. americana* tissue (Table 4), suggesting a dilution effect of metal concentrations from high plant biomass.

Comparison over time

The use of biological indicators for long-term environmental monitoring requires that they provide reliable, yet sensitive measurements of temporal change. However, variability induced by environmental factors (water depth, exposure to current and light intensity, among others) introduces the possibility that the wrong conclusion may be reached if these factors are not accounted for or if an unbalanced sampling design is used. The robustness of conclusions reached with *V. americana* was assessed by comparing 1994 and 1996 metal concentrations measured in the plant's aboveground tissues at the four downstream sites, using four types of statistical analyses. The conclusions drawn from the four types of analyses were then compared to assess the smallest reliably detectable difference in metal concentration between years (Table 5).

The method for which data had initially been gathered (one-way ANOVA balanced design for depth) yielded significant differences between 1994 and 1996 for all elements except Cd and Ni. Decreases were observed for Fe, Mn, Pb, Zn, and N; Cr and P increased. Differences >25% were easily detected whereas differences as small as 16% were found to be significant.

These conclusions were contrasted with those obtained after original data were altered to compare shallow-water (<2 m) samples collected in 1994 with deepwater (>2 m) samples collected in 1996. Such imbalance in sample depth representation (Table 5, second column) altered the conclusions yielded by the analysis for five of the nine metals (Cd, Fe, Mn, Ni, Zn), with differences as high as 27–37% (Fe, Mn, Zn) deemed not significant. Conclusions based on two other approaches were also examined using pairwise comparisons of average concentrations for each depth and site (Table 5, third column) and for site only (Table 5, fourth column). These analyses yielded increasingly conservative conclusions, likely related to their smaller number of degrees of freedom. These last two analyses reduced to four and two, respectively, the number of elements showing significant differences between years. Pb (57% difference) and N (16% difference) were the only elements for which the same conclusions were reached, regardless of the statistical method of analysis selected. The consistency of conclusions with different statistical methods appeared to be determined by low sampling variability rather than by the magnitude of change (Table 5), thus further emphasizing the need for depth-balanced sampling design.

The same exercise was carried out for the analysis of long-term (20 years) changes between 1976 (Béland and Demers 1977) and the 1990s by comparing conclusions yielded by two different statistical approaches. First, metal concentrations were compared using one-way ANOVA restricted to measurements made in the fluvial corridor downstream of Montreal (four sites). This analysis showed that concentrations recorded in plant tissue in 1976 were significantly higher than they were in 1994 and 1996 for Cd, Cr, Pb, N, and Zn; significant increases in Ni and P were also observed (Fig. 4). The second approach used pairwise (n = 6) comparisons by sector (lakes Saint-François, des Deux Montagnes, Saint-Louis, fluvial corridor north and south, and Lake Saint-Pierre) with all available data (Béland and Demers 1977; St-Cyr and Campbell 1994; this study). This analysis (results not shown) confirmed the conclusions for Cd (63% decrease), Cr (83% decrease), Pb (83% decrease), Zn (45% decrease), N (22% decrease), and Ni (33% in-

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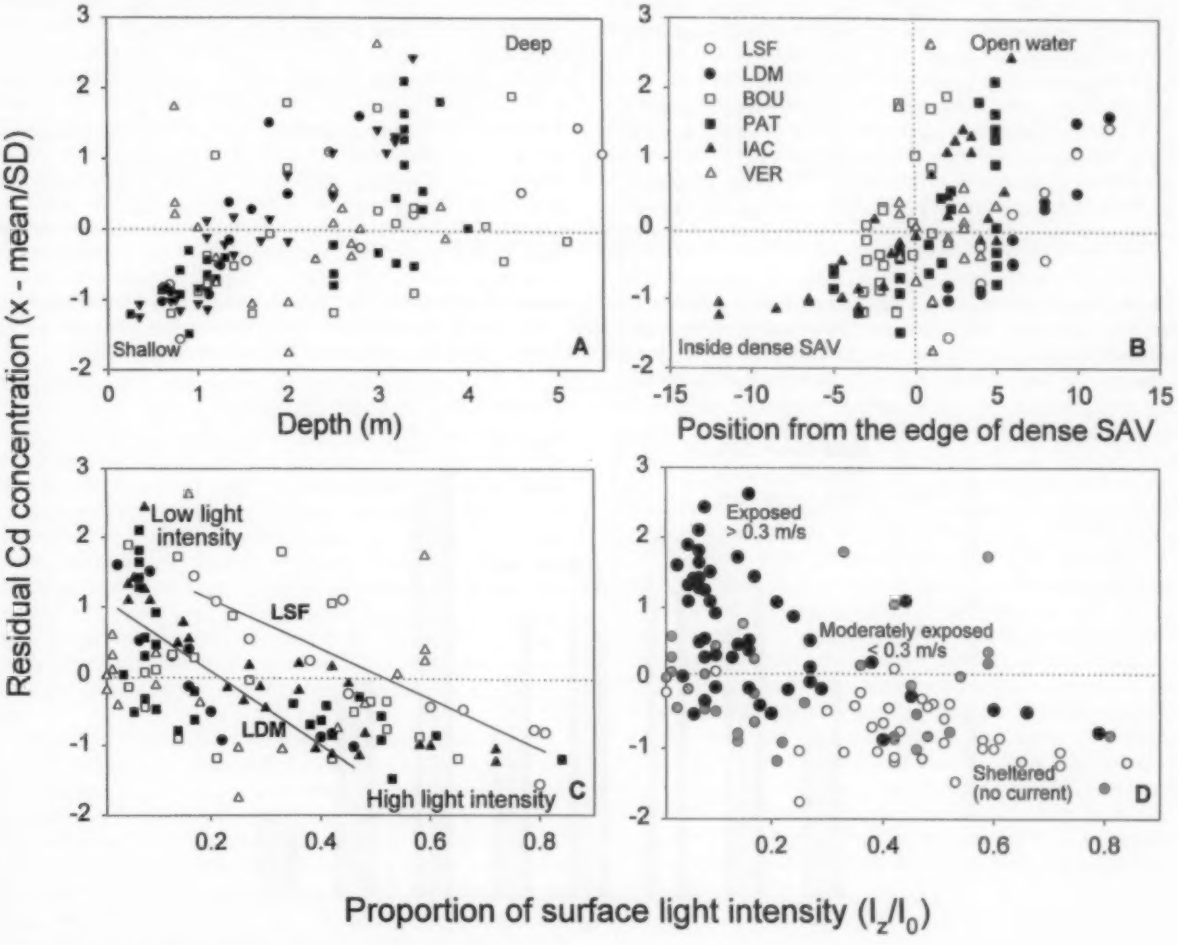
Fig. 3. Relationship between residual Cd concentrations ((individual values (x) – mean)/SD) in aboveground tissues of *V. americana* and (A) water depth, (B) position relative to the edge of dense SAV bed, and (C and D) proportion of surface light intensity. Symbols in Figs. 3A–3C refer to individual sites identified in the legend: LSF, Lake Saint-François; LDM, Lake des Deux-Montagnes; BOU, Boucherville; PAT, Pointe aux Trembles; IAC, Île aux Cerfeuils; VER, Verchères. Sites in St. Lawrence River waters (open symbols) are distinguished from sites in Ottawa River waters (solids symbols). Individual regression lines for lakes Saint-François and des Deux-Montagnes (Fig. 3C) coincide with a significant interaction term with water mass (see also Table 4). Figure 3D presents the same data as Fig. 3C, with samples identified according to exposure to current: sheltered (open circles), moderately exposed (grey circles), and exposed sites (solid circles).



crease), but yielded different conclusions for Cu and P. These results suggest that differences >35% are not overly sensitive to unbalanced sampling design.

Discussion

By monitoring metal concentrations in tissues of living organisms, we gain immediate, direct information on the biologically available metal concentrations present in the environment. In some respects, *V. americana* approaches the definition of the ideal indicator organism: it is ubiquitous in the St. Lawrence River: individual plants annually produce a large biomass of leaves and below-ground tissues, and the species tolerates a wide range of sediment types, current speed, and light intensity. Its tolerance for low light intensity allows *V. americana* to occur in shallow water as the understory layer to canopy-forming *Myriophyllum*, *Potamogeton*, and *Elodea* (Titus and Adams 1979) and also to dominate in deep water to the limit of plant distribution. In the St. Lawrence River, ambient light intensity is likely the primary factor limiting this plant's growth, since total N and P concentrations in *V. americana* tissues (Table 3) are well above the minimum requirements for maximum plant growth (Gerloff and Krombholz 1966).

Nevertheless, in other respects, *V. americana*'s suitability as an indicator is also limited by its broad distribution, which increases the variability in metal concentrations in its tissue. Small-scale (within-site) variations in tissue metal concentrations were observed in response to a combination of depth, exposure to current, and light intensity gradient.

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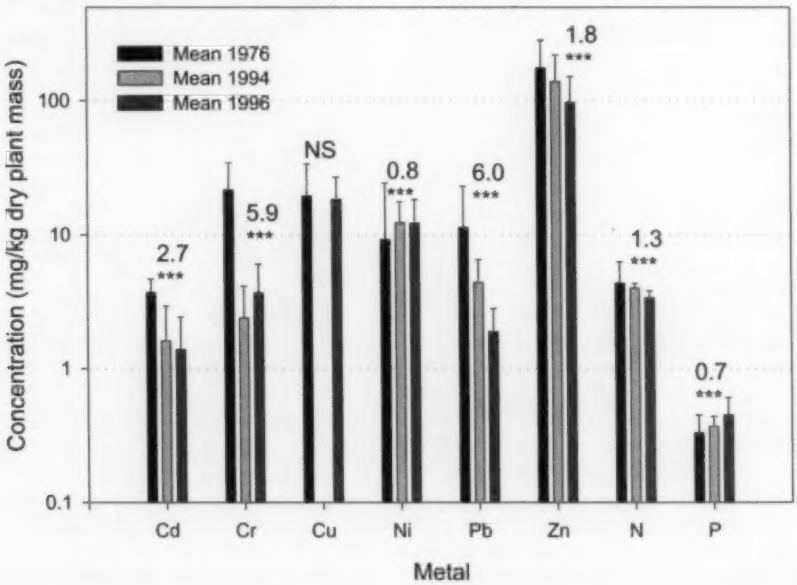
Table 5. Results of the comparison of average metal concentration in aboveground tissues of *V. americana* sampled in 1994 and 1996 at the four sites located downstream of the Greater Montreal urban area, using a one-way ANOVA among sites (columns 1 and 2), a paired *t*-test between depth strata at each site (column 3), or a paired *t*-test between sites (column 4).

	(1) One-way ANOVA between years, balanced depth design					
Metal	Mean 1994 (n = 53)	Mean 1996 (n = 40)	% change	(2) One-way ANOVA between years, unbalanced depth design, 1994 <2 m (n = 25), 1996 >2 m (n = 22)	(3) t-test between years with paired depth and sites (n = 13)	(4) t-test between years with paired sites (n = 4)
Cd	1.61^{ns}	1.39	14	1994 < 1996***	ns	ns
Cr	2.39**	3.67	54	1994 < 1996***	1994 < 1996*	ns
Fe	1818**	1152	37	ns	ns	ns
Mn	2467**	1801	27	ns	1994 > 1996*	ns
Ni	12.32^{ns}	12.21	1	1994 < 1996***	ns	ns
Pb	4.40***	1.89	57	1994 > 1996***	1994 > 1996***	1994 > 1996***
Zn	139**	97	30	ns	ns	ns
N	4.00***	3.38	16	1994 > 1996***	1994 > 1996**	1994 > 1996*
P	0.36***	0.45	23	1994 < 1996***	ns	ns

Note: Significance of the differences: ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001.

Fig. 4. Comparison of average metal concentrations (mean ± SD) in submerged macrophytes in the St. Lawrence River fluvial corridor in 1976 (solid bars), 1994 (dark grey bars), and 1996 (light grey bars). The level of significance of the difference between years is indicated for each element. ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001 for each metal. The ratio of concentrations between 1976 and 1996 is reported for significant differences.



Such variability can easily obscure differences or lead to erroneous conclusions regarding temporal changes if sampling design does not take environmental (light, depth, current, plant biomass) gradients into account. Results from this study showed that metal concentrations in the aboveground tissues of *V. americana* could vary within a single site by a factor of 3–12, depending on local conditions. Concentrations of Cd and Cr exhibited the largest within-site variability. Several mechanisms, acting alone or in concert, may be advanced to explain such gradients in metal concentration, although ecological studies such as this one do not allow any one in particular to be identified.

First, differential growth rates under a broad range of light availability may result in higher metal concentrations in plants growing slowly, under low light intensity. Depending on sites, light limitation in the St. Lawrence River may result from water depth, turbid, or highly coloured waters. Conversely, high plant biomass corresponded to low metal concentrations (Table 4), thus suggesting a dilution of metals under high plant production levels.

Second, differences in the plants' absolute metal concentration could result from differences in metal bioavailability, such as was observed in lakes Saint-François and des Deux Montagnes (Fig. 3C). Although absolute metal concentrations were significantly lower in Lake Saint-François (Table 3), the relative metal concentration in *V. americana* for a

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given light intensity was always higher in Lake Saint-François than in Lake des Deux Montagnes (Fig. 3C); that is, for two plants with the same growth rate and exposed to the same ambient metal concentration, the plant growing in Lake Saint-François would exhibit a higher metal uptake than the one in Lake des Deux Montagnes. This difference was evident for Cd, Mn, Ni, and Zn (Table 4) and may result from the differences in pH, Fe concentrations, redox potential, and (or) total organic C (Campbell et al. 1985) between the St. Lawrence and Ottawa rivers (Table 1).

Third, plants subjected to the direct effects of the current may assimilate more metals in their aboveground tissues owing to the reduction of boundary layer thickness. For a given light intensity, plants exposed to currents exhibited higher relative metal concentrations than plants growing under sheltered conditions (Fig. 3D). However, since depth, position within the macrophyte bed, exposure to current, light intensity, and total plant biomass strongly covary under field situations, it was not possible to distinguish their individual contribution to this gradient. Controlled laboratory experiments are required to investigate these hypotheses further.

Sensitivity of *V. americana* as an indicator

Regardless of the mechanisms responsible for the observed gradients, our findings suggest that the environmental conditions to which indicator organisms are subjected must be taken into account within a monitoring program in order to produce accurate results and for optimal performance of the indicator. Such variations, if they are ignored, may obscure differences or induce erroneous results whereas their inclusion in the sampling strategy can confer additional sensitivity and power to the analysis. The results of our comparison of metal concentrations among sites, and after a 2- and a 20-year span, are good examples of the practical consequences of appropriate sampling design and statistical analysis.

Significant differences in metal concentrations were observed among sites, reflecting the combined influence of water masses, local sources of pollution, and individual site morphology. *Vallisneria americana* sampled at sites under the influence of St. Lawrence River green waters exhibited lower metal concentrations in tissue than did those at sites influenced by Ottawa River brown waters. However, in contrast with metal concentrations in water, *V. americana* tissues did not always reflect the downstream increase in metals within the same water mass. This discrepancy could result from the fact that metal concentrations in plant tissues may respond to additional factors such as local contaminant sources and (or) site morphology (Estabrook et al. 1985). For example, the high metal concentrations in plants from the Pointe aux Trembles site could result from the cumulative exposure to local sources of metals discharged from the harbour into the Ottawa River brown waters flowing along the south side of the Island of Montreal. In contrast, the low metal concentrations observed in Verchères could result from the relative isolation of the channel from the main water current. These differences among water masses and sites suggest that metal concentrations in aboveground tissues of *V. americana* are sensitive to a wide range of environmental factors.

The comparison of metal concentrations at the four sites in the fluvial corridor in 1994 and 1996 yielded different conclusions, whether the analyses were carried out for two groups corresponding to each sampling year, for 13 pairs of depth intervals within each site, or for four pairs of sites. The number of elements showing significant differences was largely determined by the number of degrees of freedom of the analysis: only the most robust differences (Pb and N) persisted in all types of analyses. The use of a depth-balanced sampling design for the comparison of 1994 and 1996 revealed a significant decrease in Pb, Fe, Mn, Zn, and N in aboveground tissue of *V. americana* and an increase in Cr and P. By contrast, the use of an unbalanced depth design modified many of the conclusions reached previously and decreased the sensitivity of the measure of change from about 20 to >35%. This information is crucial to optimizing monitoring frequency, sampling design, and the number of samples required to highlight future spatial or temporal differences.

Over the last 20 years, significant reductions in metal concentrations in SAV have occurred for Cd (by a factor of 2.7), Cr (factor of 5.9), Pb (factor of 6), and Zn (factor of 1.8). Although the exact magnitude of the decrease is subject to methodological uncertainties, our conclusion as to a temporal decrease in metal concentrations in the St. Lawrence River is supported by sediment core data (Carignan et al. 1994). Sediment records from lakes Saint-François and Saint-Louis indicate that maximum metal concentrations occurred in the 1970s, followed by a period of decline for Cd (factor of 3), Cr (factor of 1.5), Cu (factor of 1.5), Pb (factor of 2), and Zn (factor of 3). Sedimentary and SAV data records thus appear to agree on both the direction and the magnitude of changes in metal concentrations over a long time span. Such a decrease may result from lower metal concentrations from Lake Ontario as well as from local reductions in raw industrial and domestic sewer inflow in the Greater Montreal area. Repeat sampling at the same sites is advisable so as to follow the progressive cleanup of this area in the future. However, our findings on spatial variations in metal concentrations in *V. americana* in the St. Lawrence River imply that particular care must be taken in the design of future monitoring programs if this species is to be used as a biological indicator of long-term reductions in metal concentrations.

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References

Béland, J., and Demers, R. 1977. Teneur en substances toxiques dans les plantes aquatiques et les facteurs limitant leur croissance. Rapp. tech. No. 3, Comité d'étude sur le fleuve St-Laurent, Montreal, Que.

Campbell, P.G.C., Tessier, A., Bisson, M., and Bougie, R. 1985. Accumulation of copper and zinc in the yellow water lily, *Nuphar variegatum*: relationships to metal partitioning in the adjacent lake sediments. Can. J. Fish. Aquat. Sci. **42**: 23–32.

Carignan, R., Lorrain, S., and Lum, K. 1994. A 50-yr record of pollution by nutrients, trace metals, and organic chemicals in the St. Lawrence River. Can. J. Fish. Aquat. Sci. **51**: 1088–1100.

Cossa, D., Pham, T.-T., Rondeau, B., Quémerais, B., Proulx, S., and Surette, C. 1998. Bilan massique des contaminants chimiques dans le fleuve Saint-Laurent. Sci. Tech. Rep. ST-153, Environment Canada, Quebec Region, Environmental Conservation, St. Lawrence Centre, Montreal, Que.

Désilets, L., and Langlois, C. 1989. Variabilité spatiale et saisonnière de la qualité de l'eau du fleuve Saint-Laurent. Environment Canada, Quebec Region, Inland Waters Directorate, Conservation and Protection, Montreal, Que.

Dykyjová, D. 1973. Content of mineral macronutrients in emergent macrophytes during their seasonal growth and decomposition. *In* Ecosystem study on wetland biome in Czechoslovakia. *Edited by* S. Hejný. Czechoslovak IBP/PT-PP Rep. No. 3, Czechoslovak Academy of Sciences, Czechoslovak National Committee for the International Biological Programme, Třebön, Czechoslovakia. pp. 163–172.

Environment Canada. 1979*a*. Nitrogen: organic–particulate. NAQUADAT method No. 07901, Analytical Methods Manual, Inland Waters Directorate, Water Quality Branch, Ottawa, Ont.

Environment Canada. 1979*b*. Method for the analysis of total phosphorus. NAQUADAT method No. 15050, Analytical Methods Manual, Inland Waters Directorate, Water Quality Branch, Ottawa, Ont.

Environment Canada. 1994*a*. Méthode d'analyse des métaux (Cr, Cu, Fe, Mn, Ni, Zn) dans les plantes aquatiques. Analytical scheme TM-AA/T8P, Environment Canada National Laboratory, Burlington, Ont.

Environment Canada. 1994*b*. Méthode d'analyse des métaux (Cd, Pb) dans les plantes aquatiques. Analytical scheme TM-AAFG/T2-P, Environment Canada National Laboratory, Burlington, Ont.

Estabrook, G.F., Burk, D.W., Inman, D.R., Kaufman, P.B., Wells, J.R., Jones, J.D., and Ghosheh, N. 1985. Comparison of heavy metals in aquatic plants on Charity Island, Saginaw Bay, Lake Huron, U.S.A., with plants along the shoreline of Saginaw Bay. Am. J. Bot. **72**: 209–216.

Gerloff, G.C., and Krombholz, P.H. 1966. Tissue analysis as a means of nutrient availability for the growth of angiosperm aquatic plants. Limnol. Oceanogr. **11**: 529–537.

Gommes, R., and Muntau, H. 1981. Variations saisonnières de la composition chimique des limnophytes du Lago Maggiore. Mem. Ist. Ital. Idrobiol. Dott Marco Marchi, **38**: 309–330.

Greger, M., and Kautsky, L. 1992. Use of macrophytes for mapping bioavailable heavy metals in shallow coastal areas, Stockholm, Sweden. Appl. Geochem. **2**: 37–43.

Hudon, C. 1997. Effects of water-level fluctuations on St. Lawrence River aquatic vegetation. Can. J. Fish. Aquat. Sci. **54**: 2853–2865.

Hudon, C., and Lalonde, S. 1998. Caractérisation de la biomasse et teneur en métaux des herbiers du Saint-Laurent (1993–1996). Sci. Tech. Rep. ST-174, Environment Canada, Quebec Region, Environmental Conservation, St. Lawrence Centre, Montreal, Que.

Hudon, C., and Sylvestre, A. 1998. Qualité de l'eau en aval de Montréal 1994–1996. Sci. Tech. Rep. ST-170, Environment Canada, Quebec Region, Environmental Conservation, St. Lawrence Centre, Montreal, Que.

Jackson, L.J., Rasmussen, J.B., Peters, R.H., and Kalff, J. 1991. Empirical relationships between the element composition of aquatic macrophytes and their underlying sediments. Biogeochemistry (Dordr.), **12**: 71–86.

Miller, G.E., Wile, I., and Hitchin, G.G. 1983. Patterns of accumulation of selected metals in members of the soft-water macrophyte flora of central Ontario lakes. Aquat. Bot. **15**: 53–64.

Mudroch, A., and Capobianco, J.A. 1979. Effects of mine effluent on uptake of Co, Ni, Cu, As, Zn, Cd, Cr, and Pb by aquatic macrophytes. Hydrobiologia, **64**: 223–231.

Outridge, P.M., and Noller, B.N. 1991. Accumulation of toxic trace elements by freshwater vascular plants. Rev. Environ. Contam. Toxicol. **121**: 1–63.

Ray, S.N., and White, W.J. 1976. Selected aquatic plants as indicator species for heavy metal pollution. J. Environ. Sci. Health Part A Environ. Sci. Eng. **11**: 717–725.

Ray, S.N., and White, W.J. 1979. *Equisetum arvense* — an aquatic vascular plant as a biological monitor for heavy metal pollution. Chemosphere, **3**: 125–128.

Rondeau, B. 1993. Qualité des eaux du fleuve Saint-Laurent 1985–1990. Tronçon Cornwall–Québec. Sci. Tech. Rep. EN 153-30/1990, Environment Canada, Quebec Region, Environmental Conservation, St. Lawrence Centre, Montreal, Que.

Smith, C.S., James, W.F., Barko, J.W., and Eakin, H.L. 1995. Studies of water movement in vegetated and unvegetated littoral areas. Tech. Rep. A-95-3, U.S. Army Corps of Engineers, Vicksburg, Miss.

Sprenger, M., and McIntosh, A. 1989. Relationship between concentrations of aluminum, cadmium, lead, and zinc in water, sediments, and aquatic macrophytes in six acidic lakes. Arch. Environ. Contam. Toxicol. **18**: 225–231.

St-Cyr, L., and Campbell, P.G.C. 1994. Trace metals in submerged plants of the St. Lawrence River. Can. J. Bot. **72**: 429–439.

Titus, J.E., and Adams, M.S. 1979. Coexistence and the comparative light relations of the submersed macrophytes *Myriophyllum spicatum* L. and *Vallisneria americana* Michx. Oecologia (Berl.), **40**: 273–286.

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Diel variation in trawl catchability: is it as clear as day and night?

Jill M. Casey and Ransom A. Myers

Abstract: Diel variation in the catchability of over 50 species was examined using research vessel surveys conducted off the coast of Newfoundland and Labrador from 1972 to 1995. Catchability during the day and night was estimated for several seasons and geographic areas in the Northwest Atlantic using two generalized linear models. In general, species exhibiting diel vertical migrations, such as redfish (*Sebastes* sp.), northern sand lance (*Ammodytes dubius*), and haddock (*Melanogrammus aeglefinus*), were caught in higher proportions during the day. Nonmigrating species, such as flatfish, skate, and sculpin, which rely on visibility of the trawl as a means of escapement, were caught in higher proportions during the night. Analysis of the effect of depth indicated that catchability during the day, relative to the night, increased significantly with depth for 21 species. We demonstrate that the accepted methods of estimating standard errors for generalized linear models are not valid for survey trawl data and suggest alternative methods.

Résumé : De 1972 à 1995, nous avons mené des campagnes de recherche au large de la côte de Terre-Neuve et du Labrador afin d'étudier les variations nycthémérales de la capturabilité de plus de 50 espèces. Pour estimer la capturabilité de jour et de nuit de ces espèces, nous avons utilisé deux modèles linéaires généralisés que nous avons appliqués à des données recueillies sur une période de plusieurs saisons et dans diverses régions du nord-ouest de l'Atlantique. En général, les espèces effectuant des migrations verticales nycthémérales comme le sébaste (*Sebastes* sp.), le lançon du nord (*Ammodytes dubius*) et l'aiglefin (*Melanogrammus aeglefinus*) ont été capturées en plus grande proportion le jour. Quant aux espèces non migratrices comme les poissons plats, la raie et le chabot, qui peuvent échapper au chalut lorsqu'il est visible, elles étaient capturées en plus grand nombre la nuit. Nous avons analysé l'effet de profondeur et constaté que, par comparaison à la capturabilité de nuit, la capturabilité de jour de 21 espèces augmente dans une mesure significative en fonction de la profondeur. Pour finir, nous démontrons que les méthodes acceptées pour l'estimation de l'écart-type des modèles linéaires généralisés ne sont pas valables lorsqu'on traite des données de chalutage, et nous en proposons d'autres.

[Traduit par la Rédaction]

Introduction

Many countries conduct bottom trawl surveys for the purpose of estimating abundance of commercial species. Determining the efficiency of survey gear, however, is a key component of estimating abundance and interpreting these survey results. The survey vessel, time of year, and time of day affect the survey trawl efficiency. Fish behaviour in response to physical factors, such as light intensity, has been shown to affect the efficiency of the survey gear over a 24-h period (Walsh 1991). Abundance of species exhibiting diel vertical migration would be overestimated from surveys conducted during the day as compared with surveys conducted at night, when these species would be out of the vertical range of the trawl (Michalsen et al. 1996). Increased visibility of the trawl during the day (Glass and Wardle 1989) could result in lower catches of nonmigrating species and thus abundance would be underestimated. Consequently, estimated abundance of species with variable patterns of diel catchability may be inaccurate. Analysis must be carried out to determine if diel variability in catchability exists, and if so, to correct for this difference.

Several studies of diel catchability have made use of designed experiments in which a predetermined number of tows was carried out under controlled conditions (Walsh 1991; Walsh and Hickey 1993; Engs and Soldal 1992). While important information on fish behaviour in the vicinity of survey gear has been obtained, the number of tows completed have often been too few to give precise estimates. Furthermore, the results may not take into account regional or seasonal changes in catchability.

The purpose of this paper is to present a simple method that allows the relative efficiency of fishing gear during the day and night to be rigorously estimated using data routinely collected during research surveys. Our approach combines data from many years and several geographic areas.

A further motivation for this work is to provide correction factors for older research surveys of the Canadian Department of Fisheries and Oceans, many of which were conducted almost exclusively during daylight hours. As data collected from commercial vessels are thought to be unreliable, more emphasis is being placed on research vessel surveys. Correction factors for diel variation in trawl catchability are essential in determining absolute abundances from these surveys.

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Methods

Data

Research surveys have been conducted by the Canadian Department of Fisheries and Oceans off the coast of Newfoundland since 1946 (Templeman 1966). Prior to 1972, however, surveys were primarily conducted during daylight hours. We examined stratified random surveys conducted around the Island of Newfoundland and the coast of Labrador (Pitt et al. 1981) from 1972 (when both day and night surveys were conducted in roughly equal proportion) to 1995 during which time approximately 20 000 research tows were successfully made. Only tows with a duration of 30 min were selected for this analysis. Data from seven Northwest Atlantic Fisheries Organization (NAFO) subdivisions (Fig. 1), each with different bottom topographies, were included in this study. Seasons of the surveys are given in Table 1.

Species were selected for analysis if they occurred in more than 100 tows, and only strata in which individual species were caught in more than five tows were included. Sunrise and sunset were determined using the day of year and latitude of the tows (Brock 1981). Tows that occurred within 1 h of sunrise and sunset were excluded so that successive tows would not be compared (e.g., the last tow of the night and the first tow of the day).

Model

Model 1

Consider a survey in year y, in which C_{ysl} fish are caught in stratum s during the time of day (i.e., day or night) l. The catchability associated with time of day l is S_l, where the catchability will be scaled so that the catchability during the night will be 1. We construct a simple model in which an equal proportion of fish will be in each stratum in all years. The expected value of the catch, $E[C_{ysl}]$, is

$$E[C_{ysl}] = N_y P_s S_l$$

where N_y is the number of fish in the population in year y, P_s is the proportion of fish stratum s, and S_l is the combined availability and vulnerability of each species to the survey gear (or catchability) associated with time of day, l. The simplest model for the probability of catching C_{ysl} fish is a Poisson distribution. This is not a realistic model, however, because fish usually aggregate (i.e., in schools) and, as such, are not captured independently. Also, habitat within a stratum is not equally suitable. An overdispersed, i.e., extra-Poisson model, is preferred in which overdispersion is modeled using a scale factor for the variances (McCullagh and Nelder 1989). The scale factor only affects the variance but not the parameter estimates. The data can be analyzed in terms of a generalized linear model (GLIM) with a log link.

The main assumption of this model is that an equal proportion of fish will be in each stratum in all years. This may not be a valid assumption, however, as it is unlikely that the distribution of fish will remain constant from year to year.

Model 2

To remove the assumption of a constant stratum and year effect, the data considered will be restricted to strata within years in which at least one day and night tow has occurred.

Let C_{ysd} be the total catch in numbers during the day in a particular year and stratum, and C_{ys} be the total catch for the day and the night in the same year and stratum. T_{ysd} and T_{ysn} will be the corresponding number of day and night tows, respectively. Let the probability that a fish caught in a year, y, and stratum, s, is caught during the day be p_{ys} and $1 - p_{ys}$ for night catches. If there is no difference between day and night catchability, then we would expect

$$\frac{p_{ys}}{1-p_{ys}} = \frac{T_{ysd}}{T_{ysn}}$$

We are interested, however, in whether or not a different proportion of fish are caught during the day and night, and so the catchability term, S_d, is multiplied to the right-hand side of the above equation. After a log transformation we have

$$\log\left(\frac{p_{ys}}{1-p_{ys}}\right) = \log(S_d) + \log\left(\frac{T_{ysd}}{T_{ysn}}\right)$$

The left side of the equation represents the logit transformation of p_{ys}. The term on the far right is the offset (Mccullagh and Nelder 1989), a known quantitative variate, which will account for the number of day and night tows. The log of the catchability during the day, S_d, is the intercept and will be estimated.

If fish of a given species are captured independently, and if the probability of catching a fish during the day is constant for individuals of that species, then the probability of catching a fish during the day in a particular year and stratum (C_{ysd}), given the total number of fish caught in that year and stratum (C_{ys}), is binomial. Overdispersion must also be taken into account with model 2. An extra-binomial model is preferred in which overdispersion is modeled using a scale factor for the variances (McCullagh and Nelder 1989). A GLIM with a logit link and an offset is used to analyze the data. The method of programming is given in the Appendix.

This model also makes a potentially unrealistic assumption that the same proportion of fish will remain in a stratum during the survey period for a given year. Many species make seasonal migrations that take them into and out of the arbitrarily assigned strata and, as such, would not be expected to restrict their distribution during the year.

It is not clear that either of the above models is superior as both make assumptions that may not be valid for research survey data. For simplicity, model 2 was chosen for this analysis, although reference will be made to the results of model 1 as a method of comparison.

Regional and seasonal differences in diel catchability

Differences in bottom topographies associated with each NAFO area could possibly affect diel catchability. Banks within the study area range from 150–300 m in depth surrounded by troughs of 450–500 m in the north, to depths of 100 m with 150–200 m deep troughs in the south (Litvin and Rvachev 1963).

Seasonal changes in diel catchability have been suggested for various groundfish species (Beamish 1965). Since the degree of vertical migration within the water column may vary seasonally, research surveys conducted in different seasons were examined separately.

This analysis examines nine region and season combinations over which estimates of diel catchability will be compared.

Randomization tests

The reliability of confidence limits and significance tests for the day–night effect was assessed using a randomization test (Manly 1991). For each area–season combination studied (Table 1), the catchability of day tows was estimated using model 2 and the original data, with half of the data randomly assigned to the day and half to the night. Significance was determined by calculating the 95% confidence interval based on the standard errors of the GLIM estimates. The proportion of significant tests from 100 randomizations was then determined.

The randomization tests showed that the standard significance levels and standard errors of the parameter estimates were not reliable. Figure 2 shows the proportion of significant tests from the 100 randomizations for each species and region–season combina-

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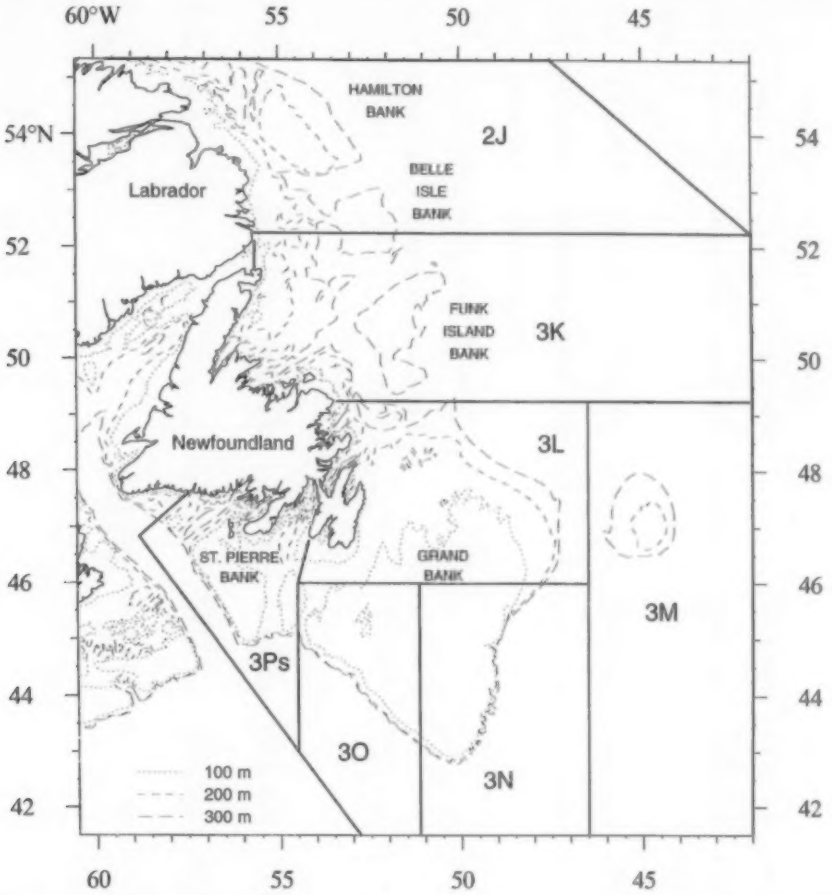
Fig. 1. Map of NAFO areas. The 100- (dotted line), 200- (short-dashed line), and 300-m (long-dashed line) isobaths are given for reference.



tion. With a significance level of 5%, the proportion of nominally significant tests should have been 0.05. For only 33% of the species and region–season combinations, however, was the proportion of significant tests less than or equal to 0.05.

Fish species that are known to school, such as capelin (*Mallotus villosus*), redfish (*Sebastes* sp.), Atlantic cod (*Gadus morhua*), and arctic cod (*Boreogadus saida*), tended to have a higher proportion of significant tests, while with solitary species, such as wolffish, the proportion of significant tests was closer to 0.05. In subdivision 3M, where the number of species and number of observations were the lowest for all subdivisions, the proportion of significant tests tended to be lower. In subdivisions with many observations, such as 3L and 3NO, the opposite was true.

Alternative estimates of reliability were constructed as the standard deviation of the mean of the parameter estimates from the 100 randomizations. These estimates of reliability in Table 1 represent the standard errors under the null hypothesis (i.e., no difference between day and night catchability) and are used in hypothesis testing (Manly 1991).

Depth effects

Depth, which has been thought to contribute to the variability in diel catchability (Pitt 1967), was examined using model 2. Tows were categorized into approximately 100-m depth classes that corresponded with the average depth of the strata included in this study (Bishop 1994). The model was run initially as a regression analysis with depth as a covariate. Since the standard significance tests are considered to be liberal, any species for which depth was not significant was eliminated. For the remaining species, estimates of reliability for the depth effect were then determined with the depth categories randomly assigned in the same proportion as would normally be observed for each species. For example, it would be unlikely that a species normally found at shallow depths would be caught in equal proportions at the greatest depths. The standard deviation of the estimates from 100 randomizations were used in hypothesis testing. Estimates of diel catchability in each depth class were then determined using model 2.

Results

Estimates of diel catchability were obtained for all region–season combinations (Table 1, Fig. 3). Negative estimates indicated higher catchabilities at night. The summary refers to estimates determined over all regions and seasons. Of the 32 species with significant differences over all regions and seasons, 12 were caught significantly more during the day and 20 were caught significantly more at night.

Estimates for the family Gadidae differed among species. Arctic cod was caught significantly more during the day in

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Table 1. Comparison of species catchability during day and night trawls.

Species	2J Fall	3K Fall	3L Fall	3L Spring	3L Summer	3M Spring	3NO Fall	3NO Spring	3Ps Spring	Summary
Clupeiformes										
Clupeidae										
Clupea harengus (herring)								2.02 (0.53)	0.81 (0.22)	0.91 (0.22)
Gadiformes										
Gadidae										
Boreogadus saida (Arctic cod)	0.24 (0.23)	–0.25 (0.16)	0.5 (0.14)	0.38 (0.17)	–0.04 (0.53)		0.95 (1.22)		–1.12 (0.24)	0.27 (0.08)
Gadus morhua (Atlantic cod)	–0.23 (0.08)	–0.15 (0.14)	–0.15 (0.1)	–0.05 (0.09)	0.09 (0.14)	1.16 (0.43)	0.26 (0.11)	0.23 (0.09)	–0.26 (0.15)	0.05 (0.05)
Gadus ogac (Greenland cod)	–0.28 (0.38)									–0.28 (0.42)
Melanogrammus aeglefinus (haddock)			–1.29 (1.08)		1 (0.97)		–1.32 (0.83)	0.87 (0.25)	0.34 (0.17)	0.51 (0.2)
Merluccius bilinearis (silver hake)							–0.99 (0.66)	0.02 (0.24)	0.37 (0.2)	0.26 (0.18)
Pollachius virens (pollock)								0.99 (0.75)	0.11 (0.36)	0.2 (0.32)
Urophycis chesteri (longfin hake)						–0.95 (0.16)	–1.55 (0.73)	–1.01 (0.26)	–0.42 (0.11)	–0.58 (0.09)
Urophycis tenuis (white hake)							–0.18 (0.46)	0.24 (0.15)	0.01 (0.09)	0.04 (0.07)
Macrouridae										
Coryphaenoides rupestris (roundnose grenadier)	–0.9 (0.65)	–1.38 (0.59)								–1.3 (0.49)
Macrourus berlax (roughhead grenadier)	0.08 (0.06)	0.09 (0.05)	0.41 (0.13)	–0.12 (0.09)	0.35 (0.14)	0.1 (0.11)	0.87 (0.48)	0.15 (0.16)		0.19 (0.04)
Nezumia bairdi (marlin-spike)	0.21 (0.24)	–0.14 (0.1)	0.11 (0.16)	0.39 (0.26)	–0.1 (0.24)	–0.27 (0.1)	–0.5 (0.31)	–0.39 (0.18)	0.04 (0.05)	–0.02 (0.04)
Moridae										
Antimora rostrata (blue hake)	–0.72 (0.42)	–0.43 (0.32)				–0.14 (0.18)				–0.27 (0.14)
Lophiiformes										
Lophiidae										
Lophius americanus (monkfish)								1.12 (1.33)	0.19 (0.12)	0.14 (0.12)
Myctophiformes										
Myctophidae										
Other (lanternfishes)	0.12 (0.22)	0.78 (0.49)	–0.25 (0.27)	3.56 (0.69)	1.84 (3.94)	–0.4 (0.43)		–1.22 (1.07)	–0.6 (0.43)	–0.18 (0.18)
Paralepididae										
Other (Barracudina)	–0.35 (0.54)	0.09 (0.3)	1.07 (0.43)	0.4 (0.25)	0.05 (0.26)				0.43 (0.3)	0.48 (0.16)

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Table 1 (*continued*).

Species	2J Fall	3K Fall	3L Fall	3L Spring	3L Summer	3M Spring	3NO Fall	3NO Spring	3Ps Spring	Summary
Synaphobranchidae										
Synaphobranchus kaupi (longnose eel)	–0.16 (0.44)	–0.28 (0.5)								–0.24 (0.37)
Myxiniformes										
Myxinidae										
Myxine glutinosa (Atlantic hagfish)									–0.65 (0.18)	–0.65 (0.2)
Notocanthiformes										
Notocanthidae										
Other (spiny eels)		–0.74 (0.38)	0.75 (0.79)	0.11 (1.17)	1.47 (0.98)	–0.58 (0.29)				–0.19 (0.24)
Perciformes										
Agonidae										
Agonus decagonus (northern alligatorfish)	–0.34 (0.14)	–0.15 (0.11)	–0.44 (0.15)	–1 (0.2)	– 0.2 (0.6)					–0.56 (0.11)
Aspidoporoides olriki (common alligatorfish)	–2.69 (0.93)		–1.06 (0.27)	–0.81 (0.48)			–2.38 (0.98)			–1.01 (0.21)
Ammodytidae										
Ammodytes dubius (northern sand lance)			1.09 (0.3)	1.78 (0.42)	1.91 (2.34)		2.43 (0.38)	2.7 (0.4)	3.58 (2)	1.59 (0.19)
Anarhichadidae										
Anarhichas denticulatus (broadhead wolffish)	–0.2 (0.05)	–0.02 (0.08)	0.16 (0.12)	–0.11 (0.23)	0.01 (0.18)	–0.14 (0.19)				–0.11 (0.04)
Anarhichas lupus (striped wolffish)	–0.09 (0.08)	–0 (0.05)	0.29 (0.11)	–0.03 (0.1)	–0.12 (0.16)	–0.87 (0.09)	–0.82 (0.32)	–0.71 (0.12)	–1.19 (0.19)	–0.29 (0.04)
Anarhichas minor (spotted wolffish)	–0.12 (0.09)	0.32 (0.15)	–0.17 (0.14)	0.07 (0.13)	0.09 (0.25)	–0.63 (0.12)		2 (0.77)		–0.09 (0.06)
Cottidae										
Artediellus sp. (hookear sculpin)	–1.73 (0.59)	–0.89 (0.13)	–2.13 (0.24)	–1.87 (0.55)						–2.02 (0.2)
Cottunculus microps (Arctic deepsea sculpin)	–0.63 (0.23)	0.23 (0.16)	–0.14 (0.78)	0.56 (0.44)						0.01 (0.11)
Hemitripterus americanus (sea raven)				0.25 (0.3)			–1.08 (0.26)	–0.52 (0.08)	–0.51 (0.08)	–0.56 (0.06)
Myoxocephalus octodecemspinosus (longhorn sculpin)			–3.55 (1.06)	–0.56 (0.55)			–1.33 (0.24)	–1.2 (0.31)	–1.84 (0.44)	–1.44 (0.19)

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Table 1 (*continued*).

Species	2J Fall	3K Fall	3L Fall	3L Spring	3L Summer	3M Spring	3NO Fall	3NO Spring	3Ps Spring	Summary
Myoxocephalus scorpius (shorthorn sculpin)			–0.78 (0.34)	0.55 (0.26)			0.43 (0.6)	–0.05 (0.25)	0.2 (0.15)	0.19 (0.1)
Triglops sp. (mailed sculpin)	–1.96 (0.38)	–1.39 (0.63)	–0.83 (0.17)	–0.78 (0.25)	–1.82 (0.29)		–0.52 (0.27)	–1.12 (0.29)	–1.13 (0.33)	–0.78 (0.14)
Cyclopteridae										
Cyclopterus lumpus (common lumpfish)	1.29 (0.42)	0.95 (0.49)	1.84 (0.67)	0.45 (0.27)				1.21 (0.24)	0.56 (0.2)	0.57 (0.14)
Eumicrotremus spinosus (spiny lumpfish)	–1.52 (0.23)		–1.21 (0.12)	–2 (0.2)	–2.13 (0.64)		–1.95 (0.54)	–1.83 (0.33)	–2.68 (0.28)	–1.54 (0.08)
Liparidae										
Other (seasnails)	–1.17 (0.87)	–1.96 (0.65)	–1.28 (0.16)	–0.95 (0.25)			–3.75 (1.59)			–1.31 (0.14)
Scorpaenidae										
Sebastes sp. (redfish)	0.65 (0.26)	0.28 (0.38)	0.59 (0.23)	0.2 (0.16)	1.01 (0.37)	0.94 (0.15)	0.99 (0.3)	1.24 (0.23)	0.63 (0.08)	0.8 (0.07)
Stichaeidae										
Eumesogrammus praecisus (fourline snakeblenny)			–1.37 (0.7)							–1.37 (0.62)
Lumpenus lumpretaeformis (snake blenny)			0.51 (0.28)				0.36 (0.44)			0.43 (0.27)
Lumpenus maculatus (shanny)			0.28 (0.32)	–1.05 (1.05)						0.12 (0.25)
Zoarcidae										
Lycodes reticulatus (Arctic eelpout)	–0.26 (0.09)	–0.46 (0.12)	–0.7 (0.11)	–0.38 (0.08)	–0.44 (0.09)		–0.12 (0.39)	–0.53 (0.15)	–0.27 (0.18)	–0.54 (0.06)
Lycodes sp. (eelpout)	0.28 (0.21)	0.19 (0.29)	–0.86 (0.68)	0.13 (0.07)				–0.35 (0.56)		0.1 (0.07)
Lycodes vahlii (Vahl's eelpout)	–0.18 (0.13)	–0.24 (0.06)	–0.19 (0.1)	–0.1 (0.11)	–0.58 (0.16)	–0.06 (0.16)	–1.79 (1.48)	0.42 (0.27)		–0.21 (0.04)
Pleuronectiformes										
Pleuronectidae										
Glyptocephalus cynoglossus (witch flounder)	0 (0.1)	0.25 (0.1)	0.19 (0.16)	0 (0.11)	–0.32 (0.1)	0.28 (0.16)	–0.59 (0.14)	–0.62 (0.13)	–0.09 (0.08)	–0.1 (0.05)
Hippoglossoides platessoides (American plaice)	0.3 (0.09)	0.16 (0.05)	0.38 (0.08)	0.39 (0.05)	0.23 (0.08)	0.21 (0.13)	0.03 (0.07)	–0.38 (0.07)	–0.29 (0.11)	0.2 (0.03)
Hippoglossus hippoglossus (Atlantic halibut)								0.26 (0.13)	0.41 (0.16)	0.34 (0.12)
Limanda ferruginae (yellowtail flounder)			–0.76 (0.19)	–0.68 (0.23)	–0.62 (0.28)		–1.02 (0.17)	–1.06 (0.11)	–0.32 (0.22)	–1 (0.1)

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Table 1 (*concluded*).

Species	2J Fall	3K Fall	3L Fall	3L Spring	3L Summer	3M Spring	3NO Fall	3NO Spring	3Ps Spring	Summary
Reinhardtius hippoglossoides (Greenland halibut)	–0.01 (0.06)	–0.07 (0.05)	0.28 (0.07)	0.32 (0.08)	–0.08 (0.1)	–0.05 (0.09)	0.78 (0.15)	0.1 (0.11)	0.02 (0.08)	0.02 (0.03)
Rajiformes										
Rajidae										
Raja radiata (thorny skate)	–1.07 (0.12)	–0.64 (0.06)	–0.8 (0.08)	–0.75 (0.09)	–0.77 (0.12)	–1.19 (0.13)	–0.74 (0.08)	–0.98 (0.12)	–1.15 (0.13)	–0.87 (0.04)
Raja senta (smooth skate)	–0.64 (0.24)	–0.61 (0.1)	–0.8 (0.36)	–0.13 (0.29)		–1.49 (0.43)	–1.54 (0.64)	–1.03 (0.36)	–0.43 (0.1)	–0.62 (0.07)
Salmoniformes										
Argentinidae										
Argentina silus (Atlantic argentine)								2.03 (0.64)	0.83 (0.2)	0.93 (0.19)
Osmeridae										
Mallotus villosus (capelin)	0.59 (1.21)	0.32 (0.19)	0.23 (0.13)	1.82 (0.2)	1.33 (0.62)		1.16 (0.78)	0.34 (0.36)	1.4 (0.74)	0.95 (0.12)
Squaliformes										
Squalidae										
Centroscyllium fabricii (black dogfish)	–0.03 (0.25)	–0.66 (0.85)				–0.47 (0.47)			0.11 (0.06)	0.1 (0.06)
Squalus acanthias (spiny dogfish)								1.39 (3.65)	–0.34 (0.51)	–0.33 (0.49)

Note: Values in parentheses are SEs computed using a randomization procedure (see text). Estimates for all regions and seasons are combined in Summary.

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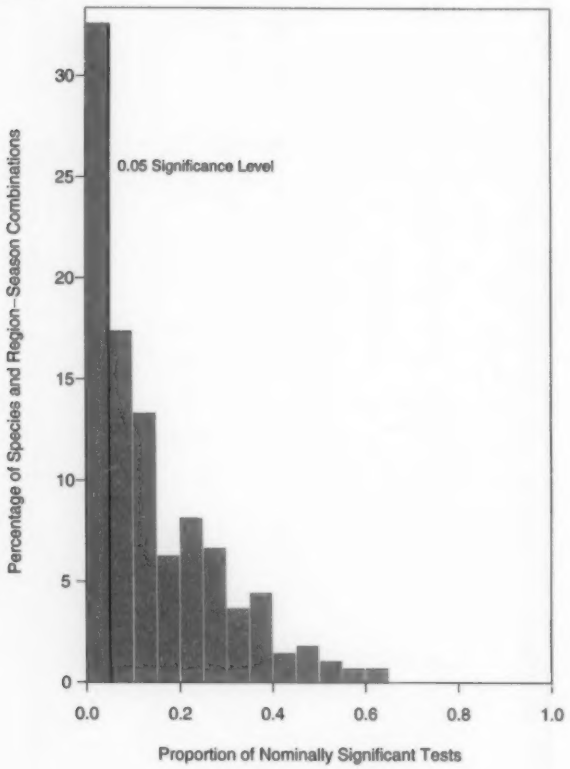
Fig. 2. Frequency of the proportion of nominally significant tests obtained from the randomizations for all species and region–season combinations (as in Table 1). The frequency is given as the percentage of species and region–season combinations (n = 266).



subdivision 3L in both the fall and spring surveys, while the estimate for subdivision 3Ps indicated a higher night catchability. The estimates for Atlantic cod were also variable with higher day catchabilities in subdivisions 3M and 3NO and a higher night catchability in subdivision 2J. Haddock (*Melanogrammus aeglefinus*) was caught significantly more during the day, while the opposite was true for longfin hake (*Urophycis chesteri*). Only arctic cod, haddock, and longfin hake had significant results across all regions and seasons.

All of the flatfish species included in this analysis had significant differences in diel catchability, although no consistent pattern among the species could be found. Witch flounder (*Glyptocephalus cynoglossus*) was caught significantly more during the night in 3L (summer only) and 3NO. Higher daytime catchability for witch founder was found in subdivision 2J only. Atlantic halibut (*Hippoglossus hippoglossus*) and Greenland halibut (*Reinhardtius hippoglossoides*) were caught significantly more during the day, while yellowtail flounder (*Limanda ferruginae*) was caught significant more at night. Estimates for American plaice (*Hippoglossoides platessoides*) differed regionally with catchability in 3NO and 3Ps spring surveys higher at night. In the north, catches were higher during the day.

Higher catchabilities during the day were found for several deepwater species, such as Atlantic argentine (*Argentina silus*) and roughhead grenadier (*Macrourus berax*), and pelagic species, such as capelin and herring (*Clupea harengus*). Redfish and northern sand lance (*Ammodytes dubius*), which have higher daytime catchabilities, have a demersal existence during the day but become pelagic feeders at night (Scott and Scott 1988).

Estimates for each species of lumpfish were consistent for all regions and seasons. Common lumpfish (*Cyclopterus lumpus*) catches, however, were significantly higher during the day, while spiny lumpfish (*Eumicrotremus spinosus*) had a significantly higher catchability at night across all regions and seasons.

For species of wolffish, sculpin, skate, and eelpout, catches were higher at night. Both striped (*Anarhichas lupus*) and spotted wolffish (*Anarichas minor*), however, also exhibited higher day catchabilities in subdivision 3L (fall only) and 3K surveys, respectively.

Parameter estimates for catchability during the day were generally not different for models 1 and 2 (Fig. 4). The only exceptions were roundnose grenadier (*Coryphaenoides rupestris*) and northern sand lance. The fact that two models, using two variations on the same data set (model 2 used a much smaller proportion of the model 1 data set), would have such similar results, suggests that the results of this analysis are believable.

Significant estimates for the effect of depth on diel catchability were obtained for 24 species (Table 2). For the majority of these species, depth was positively associated with higher catchabilities during the day relative to the night. At shallow depths, these species were more likely to caught in greater proportions during night tows, but at greater depths, catchability was likely to be higher during the day. Only three species had a negative association with depth.

Catchability was not affected by the type of vessel used over the time period. Estimates of diel catchability obtained for each region, season, and vessel combination did not differ significantly from those obtained using only region and season combinations.

Discussion

The observed diel variation in catchability may be attributed to patterns of vertical migration, and escapement associated with visibility of the trawl. Redfish and northern sand lance are known to migrate vertically at night in search of food high in the water column (Scott and Scott 1988). The high catchability during the day for both species is consistent with this type of diel vertical migration. Atkinson (1989) and Beamish (1965) found similar results for redfish in the Northwest Atlantic.

Previous studies of Atlantic cod and haddock have shown that catches were higher during the day (Engs and Soldal 1992; Walsh and Hickey 1993; Michalsen et al. 1996). Using acoustic techniques, Beamish (1965) found that both species were found in higher concentrations on the bottom during the day. Our results for haddock are consistent with these studies. For Atlantic cod, however, diel catchabilities were variable among regions. The previous studies considered surveys conducted over a small geographic area and a

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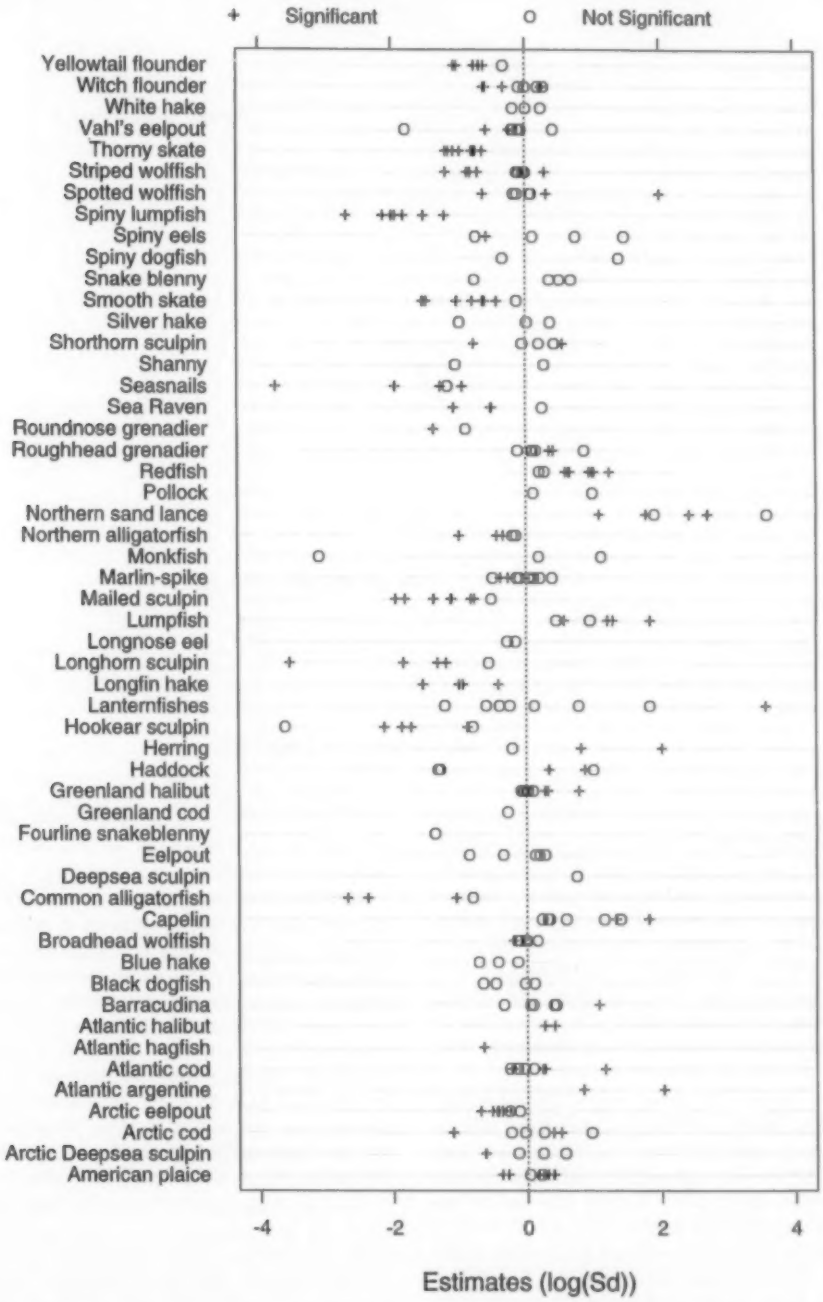
Fig. 3. Estimates of the day–night effect for all region and season combinations (as in Table 1). Negative results indicate higher catchability at night. Significant (+) and nonsignificant (o) results are differentiated, and a reference line at zero is provided for comparison.



short period of time and thus may not represent the variability of these species over their entire range.

Visibility is important to the ability to escape the trawl during daylight hours for some species (Glass and Wardle 1989), e.g., underwater video cameras have shown that some flatfish are able to avoid the trawl during the day (Walsh 1988; Walsh and Hickey 1993). In this study, however, not all flatfish showed higher catchabilities during night tows. Atlantic halibut, a deepwater species, was caught significantly more during the day indicating that this species may be migrating up into the water column at night, a previously undocumented phenomenon. The same may be true for Am-

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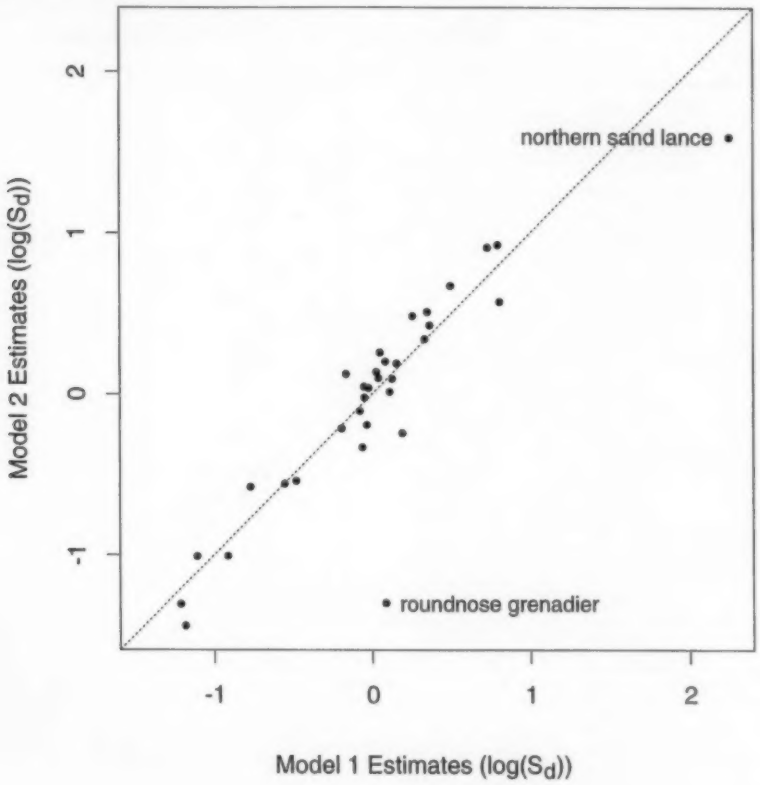
Fig. 4. Comparison of the parameter estimates combined over all regions and seasons for models 1 and 2. Each point represents an individual species. A one-to-one reference line is also given.



erican plaice found in deep water. Walsh (1991) carried out experiments on the Grand Banks and found higher catchabilities of American plaice at night, while Beamish (1965), who examined data from the Scotian Shelf and the Gulf of St. Lawrence, found the opposite. These regional differences, however, may be confounded by the effects of depth on diel catchability. In our analysis, estimates of diel catchability for American plaice were higher during the day in the northern regions which also have the deepest strata. In the south, where strata are shallower, catchabilities were higher at night.

Analysis of the influence of depth indicated that catchability during the day, relative to the night, increased significantly with depth for 21 species. When diel catchability was examined for each depth zone, the greatest differences were between the shallowest depth (<91 m) and the rest of the zones. If light does not penetrate farther than the shallowest depth, then the diel catchability should not necessarily differ beyond this depth. This increase in catchability during the day with depth may be the result of diel vertical migrations.

This study has shown that there are clear differences between day and night catchabilities for many species in the Northwest Atlantic and that depth and geography are also important factors. Size and abundance of species, although not considered in this analysis, may also be important in determining the diel variation in trawl catchability (Korsbrekke and Nakken 1997). These differences should be taken into account in the assessment of fish stocks to improve estimates of abundance.

Stratified random surveys are conducted annually for the purpose of estimating abundance of commercial species. Our analysis has shown that this data can be used to augment other studies that require the use of specified controlled experiments. Research surveys provide many replicates of data necessary for rigorous hypothesis testing and should not be overlooked as a source of valuable information. Our results will allow the time series of groundfish research surveys to be extended back 45 years, which was previously not possible because the earlier surveys occurred mostly during the day. Such an analysis is crucial for resolving major ecological issues (Greenstreet and Hall 1996).

An important general statistical issue has been raised as a result of this analysis. The standard methods for dealing with extra-Poisson and extra-binomial variation in GLIMs do not appear to provide an adequate basis for inference and hypothesis testing when dealing with stratified random research survey data. Our randomization testing clearly demonstrated that the variation in both models could not be explained by the scale factor, which inflates the binomial and Poisson variances in GLIMs. We suggest that, for research survey data and perhaps for other highly variable fisheries data, randomization testing be undertaken to dem-

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Table 2. Influence of depth on diel catchability.

Species	Estimate of the depth effect	Depth zone (m)				
		<91	92–183	184–274	275–366	367–549
American plaice	0.39 (0.03)	–0.28 (0.06)	0.42 (0.06)	0.51 (0.1)	0.24 (0.11)	0.28 (0.28)
Arctic cod	0.29 (0.12)	–0.47 (0.32)	0.35 (0.15)	0.22 (0.18)	0.93 (0.43)	
Arctic eelpout	0.38 (0.09)	–1.09 (0.13)	–0.58 (0.11)	–0.14 (0.15)	–0.38 (0.15)	
Atlantic hagfish	0.99 (0.35)		–3.33 (19.1)	–1.05 (0.48)	–0.13 (0.4)	0.21 (21.91)
Barracudina	0.56 (0.25)			–0.16 (0.43)	0.46 (0.31)	0.96 (0.26)
Capelin	–0.44 (0.13)	1.41 (0.44)	1.12 (0.2)	0.2 (0.21)	0.72 (0.43)	0.49 (9.24)
Common alligatorfish	1.06 (0.29)	–3.41 (0.69)	–1.25 (0.3)	–0.05 (0.39)	–0.11 (0.53)	
Common lumpfish	–0.52 (0.21)	1.28 (0.36)	0.34 (0.31)	0.87 (0.67)	–0.1 (0.51)	0.66 (0.34)
Hookear sculpin	0.84 (0.22)	–2.38 (0.75)	–2.18 (0.27)	–0.55 (0.3)	–0.34 (0.54)	
Longfin hake	0.46 (0.14)			–0.96 (0.32)	–0.82 (0.16)	–0.12 (0.12)
Longhorn sculpin	0.89 (0.37)	–1.48 (0.22)	–0.39 (0.47)	–1.42 (5.33)		
Longnose eel	–1.87 (0.52)				1.11 (17.02)	–0.76 (0.7)
Marlin-spike	0.15 (0.07)		–0.09 (14.34)	0.02 (0.14)	–0.27 (0.14)	0.07 (0.07)
Northern alligatorfish	0.67 (0.22)	–1.52 (3.84)	–1.17 (0.21)	–0.39 (0.31)	0.15 (0.28)	
Sea raven	0.39 (0.11)	–0.72 (0.08)	–0.35 (0.14)	0.13 (0.47)		
Seasnails	0.79 (0.23)	–4.05 (0.35)	–1.22 (0.21)	–0.84 (0.2)	–0.28 (0.27)	
Shorthorn sculpin	0.94 (0.29)	–0.22 (0.17)	0.72 (0.22)			
Smooth skate	0.32 (0.09)	–1.33 (9.36)	–0.99 (0.12)	–0.59 (0.13)	–0.3 (0.18)	–0.06 (0.23)
Spiny eels	1.79 (0.35)				–1.46 (0.71)	0.33 (0.29)
Spiny lumpfish	0.99 (0.18)	–2.51 (0.18)	–1.43 (0.12)	–0.55 (0.41)		
Striped wolffish	0.22 (0.05)	–0.77 (0.12)	–0.37 (0.08)	–0.16 (0.07)	0.01 (0.09)	–0.78 (0.56)
Thorny skate	0.14 (0.04)	–0.93 (0.1)	–0.99 (0.07)	–0.82 (0.08)	–0.44 (0.11)	–0.13 (0.17)
Vahl's eelpout	0.16 (0.06)	0.09 (6.55)	–0.49 (0.17)	–0.21 (0.08)	–0.06 (0.1)	–0.06 (0.14)
Witch flounder	0.22 (0.06)	–0.96 (0.17)	–0.24 (0.17)	–0.11 (0.1)	0.1 (0.13)	0.17 (0.11)

Note: The second column is the estimate of the slope from the regression analysis, where positive estimates indicate higher catches during the day relative to the night as depth increases. Values in parentheses are SEs under the null hypothesis (as in Table 1). Only those species with significant estimates are shown. Estimates of diel catchability in each depth zone, which correspond to average depths of the strata, are given for comparison.

onstrate the reliability of inferences. We have demonstrated, however, that by examining data from many geographical areas, the reliability of inferences can often be determined.

Acknowledgements

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References

Atkinson, D.B. 1989. Diel movements of beaked redfish and the implications of these for stratified random bottom trawl estimates of biomass and abundance. N. Am. J. Fish. Manage. **9**: 163–170.

Beamish, F.W.H. 1965. Vertical migration by demersal fish in the Northwest Atlantic. J. Fish. Res. Board Can. **23**: 109–139.

Bishop, C.A. 1994. Revisions and additions to stratification schemes used during research vessel surveys in NAFO Subareas 2 and 3. NAFO SRC Doc. No. 94/43.

Brock, T.D. 1981. Calculating solar radiation for ecological studies. Ecol. Modell. **14**: 1–19.

Engs, A., and Soldal, A.V. 1996. Diurnal variations in bottom trawl catch rates of cod and haddock and their influence on abundance indices. ICES J. Mar. Sci. **49**: 89–95.

Glass, C.W., and Wardle, C.S. 1989. Comparison of the reactions of fish to a trawl gear, at high and low light intensities. Fish. Res. (Amsterdam), **7**: 249–266.

Greenstreet, S.P.R., and Hall, S.J. 1996. Fishing and the groundfish assemblage structure in the north-western North Sea: an analysis of long-term and spatial trends. J. Anim. Ecol. **65**: 557–598.

Korsbrekke, K., and Nakken, O. 1997. Length and species dependant diurnal variation in catch rates in the Norwegian Barents Sea bottom trawl surveys. ICES C.M. W:20.

Litvin, V.M., and Rvachev, V.D. 1963. The bottom topography and sediments of the Labrador and Newfoundland fishing areas. *In* Soviet fisheries investigations in the Northwest Atlantic. *Edited by* Y.Y. Marti. Israel Program for Scientific Translations, Jerusalem. pp. 100–112.

Manly, B.F. 1991. Randomization and Monte Carlo methods in biology. Chapman & Hall, New York.

McCullagh, P., and Nelder, J.A. 1989. Generalized linear models. Monogr. Stat. Appl. Prob. Chapman & Hall, London.

Michalsen, K., God, O.R., and Ferno, A. 1996. Diel variation in the catchability of gadoids and its influence on the reliability of abundance indices. ICES J. Mar. Sci. **53**: 389–395.

Pitt, T.K. 1967. Diurnal variation in the catches of American plaice, *Hippoglossoides platessoides* Fabr., from the Grand Bank. ICNAF Res. Bull. **4**: 53–58.

Pitt, T.K., Wells, R., and Mckone, W.D. 1981. A critique of research vessel otter trawls by the St. John's Research and Resource Services. *In* Bottom trawl surveys. *Edited by* W.G. Doubleday and D. Rivard. Fisheries and Oceans Canada, Ottawa, Ont. pp. 42–61.

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Scott, W.B., and Scott, M.G. 1988. Atlantic fishes of Canada. Can. Bull. Fish. Aquat. Sci. No. 219.

Templeman, W. 1966. Marine resources of Newfoundland. Bull. Fish. Res. Board Can. No. 154.

Walsh, S J. 1988. Diel variability in the trawl catches of juvenile and adult yellowtail flounder on the Grand Banks and the effect on resource assessment. N. Am. J. Fish. Manage. **8**: 1261–1272.

Walsh, S.J. 1991. Diel variation in availability and vulnerability of fish to a survey trawl. J. Appl. Ichthyol. **7**: 147–159.

Walsh, S.J., and Hickey, W.M. 1993. Behavioural reactions of demersal fish to bottom trawls at various light conditions. ICES Mar. Sci. Symp. **196**: 68–76.

Appendix. Estimation in SAS

This appendix demonstrates how to fit the proposed model 2 to catch data from stratified random surveys.

Create a data set with seven variables per observation: species, region, season (season), number caught during the day (C_{ysd}) in a particular year and stratum, total number caught (C_{ys}) in a particular year and stratum, number of tows during the day (T_{ysd}), number of tows during the night (T_{ysn}), and time of day (daytime). A sample of what the data set should look like is given in Table A1.

The following is a brief description of the method of programmChristiane Hudoning in SAS:

```
data d1;
  infile survey; input species season Cysd Cys Tysd Tysn
    daytime;
  offset = Tysd/Tysn;
proc sort; by species season;
proc genmod; by species season;
  class daytime;
  model Cysd/Cys = daytime/link = logit dist =
    binomial offset = offset dscale noint;
```

Table A1. Sample data set to be used with model 2 (Appendix).

y	s	C_{ysd}	C_{ys}	T_{ysd}	T_{ysn}	Offset
72	383	62	65	1	1	0
72	359	73	78	1	2	–0.69
72	382	154	358	2	2	0
72	381	83	228	2	2	0
73	374	8	10	3	1	1.1
73	376	1	1	1	2	–0.69
73	354	15	22	1	2	–0.69
73	359	13	59	1	2	–0.69
74	378	129	484	1	2	–0.69
74	381	5	23	1	3	–1.1
74	379	32	296	1	2	–0.69
74	380	8	16	1	1	0
75	376	17	23	1	1	0
75	377	81	110	1	1	0
75	333	169	169	1	1	0
76	354	32	60	2	1	0.69
76	333	15	15	1	1	0
76	334	0	2	1	1	0

Note: The data represent Atlantic cod in subdivision 3NO from the spring research surveys.

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Genetic differentiation and introgression between putative subspecies of *Leuciscus soufia* (Teleostei: Cyprinidae) of the region of the Mediterranean Alps

André Gilles, Rémi Chappaz, Laurent Cavalli, Mathias Lörtscher, and Eric Faure

Abstract: Because of interspecific hybridization, there is considerable controversy over the taxonomic status of many members within the family Cyprinidae. In this study, morphological and genetic characters of *Leuciscus soufia*, a cyprinid fish of southern France and northern Italy, were examined to verify the existence of three subspecies. *Leuciscus soufia agassizi* differs from *Leuciscus soufia multicellus* in morphology, in heterozygosity at two diagnostic allozyme loci, and by distinct mitochondrial lineages. *Leuciscus s. soufia* is morphologically similar to *L. s. agassizi* but exhibits decreased enzymatic polymorphism. However, two specimens of *L. s. soufia* from the Var River were found to have mitochondria of the subspecies *multicellus*, while four Bevera River specimens of *L. s. multicellus* possessed the mitochondria of the subspecies *agassizi*. These data argue in favour of introgressive hybridization between *L. s. soufia* and *L. s. multicellus*, rejecting the hypothesis that an ancestral population was fragmented, generating three "subspecies" that retained the original polymorphism. We propose that *L. s. soufia* and *L. s. agassizi* be regrouped within the *soufia* subspecies. A calibrated molecular clock dates the separation of the remaining subspecies back to the Messinian (5 million years ago). Sea level fluctuations likely mediated their secondary contact and subsequent introgressive hybridization.

Résumé : À cause de l'hybridation interspécifique, le statut taxonomique de nombreux membres de la famille des Cyprinidés est au coeur d'un important débat. Dans le cadre de la présente étude, les auteurs ont examiné les caractères morphologiques et génétiques de *Leuciscus soufia*, cyprinidé vivant dans le sud de la France et le nord de l'Italie, afin de vérifier l'existence de trois sous-espèces. *L. s. agassizi* diffère de *L. s. multicellus* sur le plan morphologique, par l'hétérozygotie observée à deux loci de diagnostic des allozymes et par des lignées distinctes de mitochondries. *L. s. soufia* est morphologiquement similaire à *L. s. agassizi*, mais présente un polymorphisme enzymatique moindre. Toutefois, chez deux spécimens de *L. s. soufia* du Var, on a trouvé des mitochondries appartenant à la sous-espèce *multicellus*, tandis que quatre spécimens de *L. s. multicellus* de la Bevera possédaient des mitochondries de la sous-espèce *agassizi*. D'après ces données, il y aurait eu une hybridation introgressive entre *L. s. soufia* et *L. s. multicellus*, et nous pouvons rejeter l'hypothèse selon laquelle une population ancestrale aurait été fragmentée, ce qui aurait produit trois « sous-espèces » retenant le polymorphisme original. Nous proposons de regrouper *L. s. soufia* et *L. s. agassizi* dans la sous-espèce *soufia*. Une horloge moléculaire étalonnée fait remonter la séparation de l'autre sous-espèce à l'époque messinienne (5 millions d'années). Les variations du niveau de la mer ont probablement été les médiateurs du contact secondaire des sous-espèces et de leur hybridation introgressive subséquente.

[Traduit par la Rédaction]

Introduction

Speciation is of fundamental interest in biology (Dobzhansky 1951). Models of species formation have included a number of different processes, but probably the two most often cited mechanisms are allopatric and sympatric speciation (Mayr 1963). In animals, particularly in fish, several examples of sympatric (e.g., Sturmbaeur and Meyer 1992) and allopatric speciation (e.g., Lydeard et al. 1995) have been proposed. Another important mode of speciation

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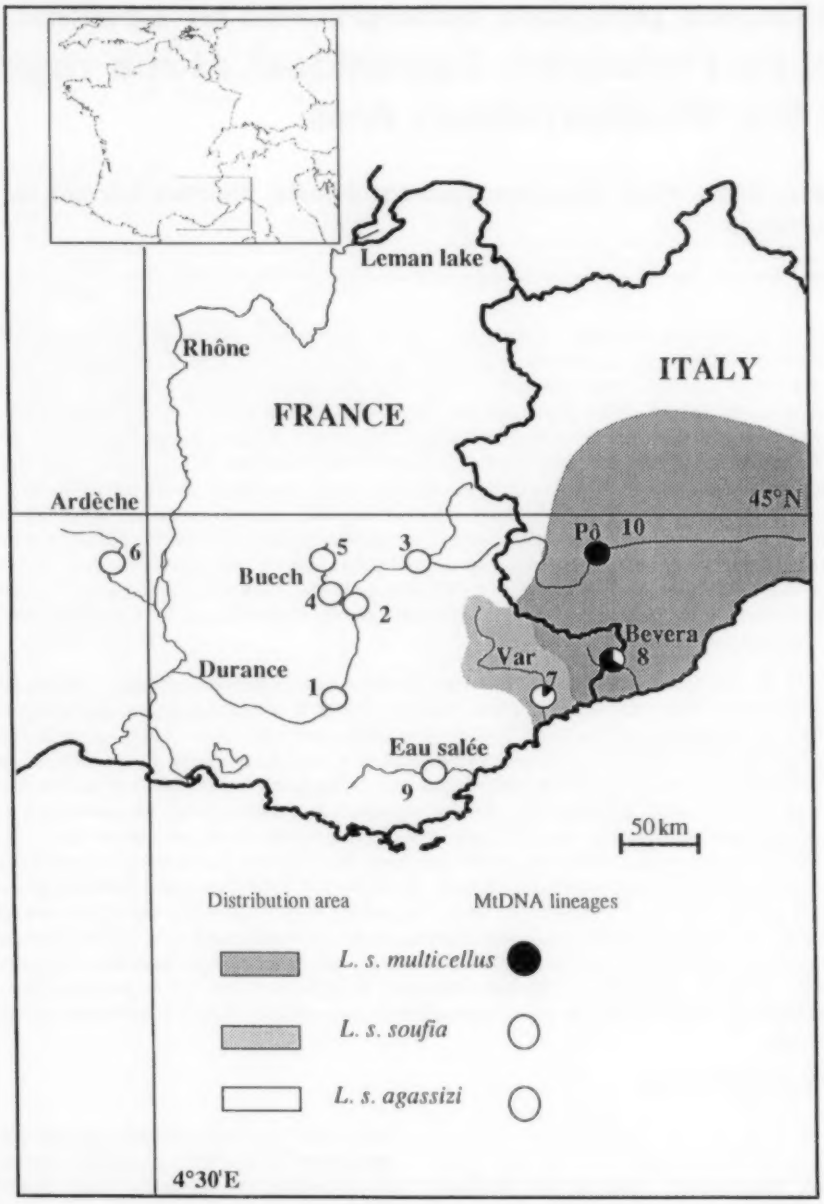
Fig. 1. Sample stations for *L. soufia* (see Table 1 for names and sample sizes) showing the repartition and the frequencies of two mtDNA lineages.



is the formation of new forms through hybridization. While all asexual fish and catostomids with an ancestral allopolyploid origin have resulted from interspecific hybridization (Avise 1994), this phenomenon has been shown occasionally in other taxa as well (Dowling and DeMarais 1993; Bernatchez et al. 1995). Despite these findings, there has been considerable controversy regarding its importance as an evolutionary process (Mayr 1963; Dowling and Secor 1997). With regard to the Cyprinidae, the phenomenon would appear to be complex, combining recent introgressions and adaptive radiation (Gilles et al. 1998) with strong hybridogenetic capacities (Brito et al. 1997). This complexity highlights the need for a systematic study of the different taxa within this family and the use of a wide range of powerful markers.

Even though few cyprinid species occur in southern Europe, several hypotheses regarding their speciation have been proposed (Darlington 1957; Banarescu 1973; Almaça 1976; Bianco 1990) and several species have been subdivided into endemic subspecies. For *Leuciscus soufia* (Risso,

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Table 1. Locality names of the sampled populations with sample sizes for allozyme, meristic, and mtDNA data.

Locality No. (see Fig. 1)	Name of locality	River system	Basin	Enzyme sample size	Meristic sample size	DNA sample size	Subspecies
1	Manosque	Durance	Rhône	24	24	12	*L. s. agassizi*
2	Sisteron	Durance	Rhône	28	28	9	*L. s. agassizi*
3	Serre Ponçon	Durance	Rhône	30	27	9	*L. s. agassizi*
4	Laragne	Durance-Buech	Rhône	44	30	10	*L. s. agassizi*
5	Lafaurie	Durance-Buech	Rhône	32	32	9	*L. s. agassizi*
6	Aubenas	Ardèche	Rhône	21	21	10	*L. s. agassizi*
7	Var	Var	Var	36	36	17	*L. s. soufia*
8	Bevera	Bevera	Roya	29	22	12	*L. s. multicellus*
9	Eau salée	Argens	Rhône	41	34	10	*L. s. agassizi*
10	Carmaniola	Pô	Pô	0	0	4	*L. s. multicellus*
	Pertuis	Durance	Rhône	14	30	4	*A. bipunctatus*

Note: The subspecies names follow the nomenclature described by Spillmann (1961).

Table 2. Multiple comparisons after a Kruskal–Wallis test.

	1. Manosque	2. Sisteron	3. Serre-Ponçon	3. Laragne	4. Lafaurie	5. Ardèche	6. Var	8. Bevera
2. Sisteron	+							
3. Serre-Ponçon	+							
4. Laragne	+		+					
5. Lafaurie		+						
6. Ardèche	+		+		+			
7. Var		+	+	+o	+	+		
8. Bevera	*	+*	*	*	*	+*	+*	
9. Eau salée	o	+		+		+	o	*

Note: Pairwise comparisons among the nine samples were made for each meristic character. Three axial characters (anal fin ray (ANA), dorsal fin rays (DOR), caudal fin rays (CAU)) and four bilateral characters (pectoral fin rays (PEC), pelvic fin rays (PEL), number and pattern of pharyngeal teeth (PT1, PT2), an total number of gill rakers in the first gill arch (GR), all situated on the left side of the fish) were counted. *Significant difference ($p < 0.05$) for the variable ANA; +, significant difference ($p < 0.05$) for the variable GR; o, significant difference ($p < 0.05$) for the variable PEC. Differences for the variables DOR, PT1, and PT2 were not significant.

1826), several controversial subdivisions have been discussed, based on diverging views of the hydrogeographic separation of the catchment areas (Spillmann 1961; d'Aubenton et al. 1971). In fact, all these authors have noted the existence of a cline, on the basis of plastic and meristic characters, from southern Germany to the Mediterranean coast. The gradual changes reported between the different "populations" along this cline provided the basis for establishing three systematic entities that have been elevated to the status of subspecies: *Leuciscus soufia soufia* (Risso 1826), *Leuciscus soufia agassizi* (CV 1844), and *Leuciscus soufia multicellus* (CBP 1837).

In the present study, we have analysed the taxonomic status of these three currently recognized subspecies as well as the factors responsible for the observed subdivision using a combined morphological, enzymatic, and mitochondrial DNA (mtDNA) approach. Due to the absence of stocking by angling associations, this species is ideal for the study of population genetics and modes of speciation in southern European cyprinids (Chappaz and Brun 1993; Gilles et al. 1998).

Materials and methods

Samples description and location

Fish were collected by electrofishing. Captured specimens were put in plastic sheaths and stored in liquid nitrogen at the capture sites. Five samples originated from the basin of the Durance River, one from the Ardèche River, and one from the Argens River, all representing *L. s. agassizi*. In addition, one sample from the Var River representing *L. s. soufia* and one from the Bevera River representing *L. s. multicellus* were analysed. From the Var River and Bevera River, only one sample was taken due to their very limited distribution in these rivers. Four specimens of *L. s. multicellus* from the Pô Basin (Fig. 1; Table 1) were added for the mtDNA study.

As an outgroup, we chose *Alburnoides bipunctatus*, which belongs to the subfamily Alburninae, another member of the family Cyprinidae. The subfamily Alburninae is close to the Leucicinae, which includes *L. soufia*. In addition, *A. bipunctatus* exhibits futher attributes that make it an appropriate outgroup. It has colonized the Rhône Basin only recently, which limits its polymorphism and possible hybridization events.

Morphological identification

The interest and importance of studies of meristic characters have been clearly demonstrated both for fish in general (Ward and Elliott 1993) and for cyprinids in particular (Dowling and DeMarais 1993). For this analysis, three axial characters (anal fin rays (ANA), dorsal fin rays (DOR), caudal fin rays (CAU)) and four bilateral characters (pectoral fin rays (PEC), pelvic fin rays (PEL), number and pattern of pharyngeal teeth (PT1 and PT2), and total number of gill rakers in the first gill arch (GR), all situated on the left side of the fish) were counted. The meristic data were analysed using the programs STATVIEW© and PHYLIP (Felsenstein

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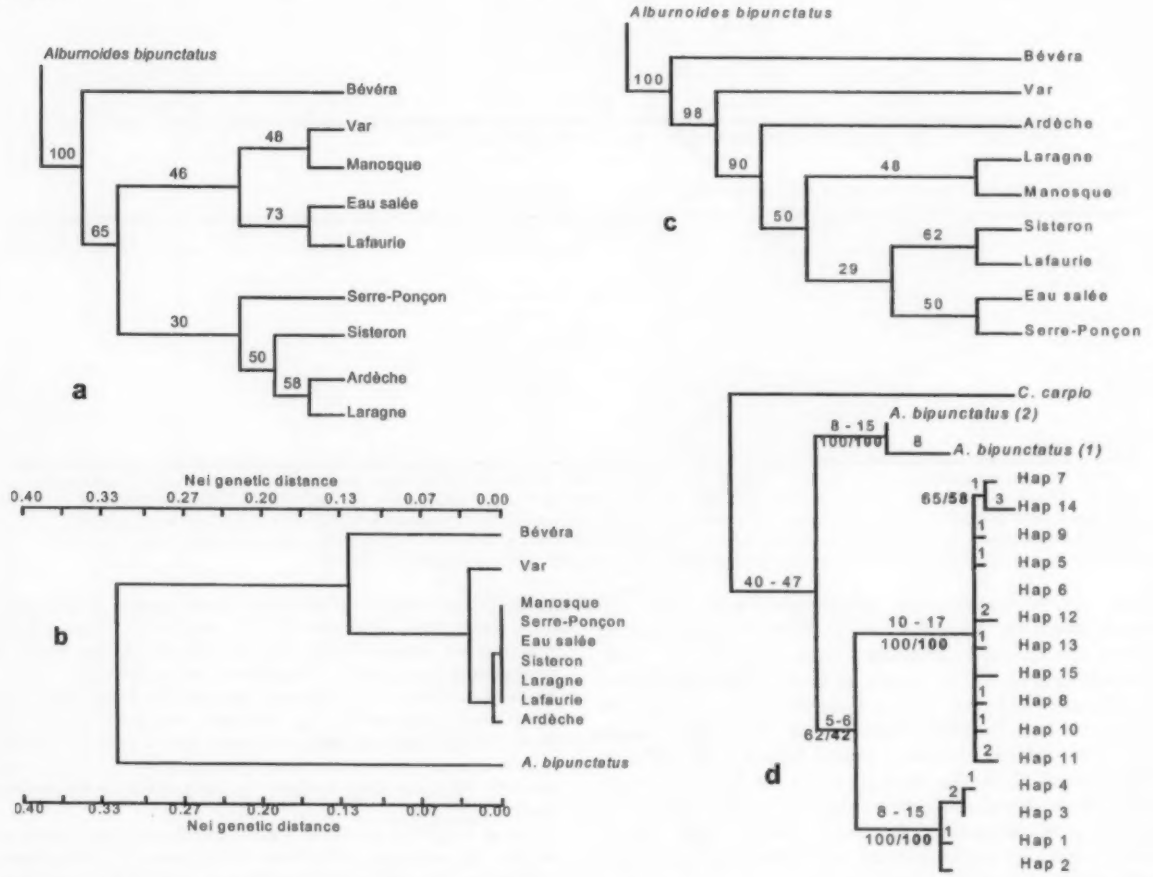
Fig. 2. (*a*) Phenogram based on 100 bootstrapped data sets of the eight meristic characters using Cavalli-Sforza and Edwards' distance and the UPGMA clustering algorithm. (*b*) Nei genetic distance UPGMA phenogram based on data from all 32 allozyme loci. The Farris coefficient *F* was 0.87 and the cophenetic correlation was 0.970. (*c*) Phenogram based on Cavalli-Sforza and Edwards' distance using the UPGMA clustering algorithm on 100 bootstrapped data sets using only the 17 polymorphic loci. (*d*) Bootstrap analyses carried out with 1000 iterations using a fragment of the 16s rDNA among 15 haplotypes of *L. soufia* and two outgroup species, using both parsimony (branch lengths are presented above corresponding branches and bootstrap values are presented below) and neighbour-joining on a matrix of nucleotide differences (bootstrap values in bold). The PAUP exhaustive seach yielded 48 equiparsimonious trees of 110 steps, with a consistency index of 0.891 and a retension index of 0.913. We show one of these most parsimonious trees, with several multifurcations due to zero branch lengths between sequences. The same topology was found when constructing a strict consensus tree based on all equiparsimonious trees, as well that found in the majority of bootstraps trees. See Table 7 for the origin of haplotypes.



1989). Of the seven meristic characters recorded, none corresponded to the criteria for performing an ANOVA and were therefore analysed with Kruskal–Wallis rank tests. Variable characters for which the Kruskal–Wallis test indicated heterogeneity for the whole set of samples were analysed using multiple comparisons of mean rank following the approach of Tomassone et al. (1993).

Allozyme analysis

For electrophoresis, between 0.5 and 1 g of muscle tissue was homogenized in extraction solution (15% (w/v) sucrose, Tris–HCl buffer (50 mM, pH 7), 10^{-6} g (w/v) of bromophenol blue) according to the method of Wynne et al. (1992). After 8 min of centrifugation, the clear supernatant was divided into microballs and preserved in a freezer at –80°C. Electrophoresis was carried out using Helena© cellulose acetate plates. Locus nomenclature (Gilles et al. 1996) follows Shaklee et al. (1990). We analysed the genetic data using heterozygosity and *F* statistics, or improved *F*, *f*, and θ coefficients of the various samples using the programs BIOSYS-1 (Swofford and Selander 1981), Genepop 1 (Raymond and Rousset 1995), and PHYLIP.

Characterization of mtDNA

For mtDNA analysis, we studied 102 specimens including some that exhibited an unusual shape and (or) rare allozyme alleles likely to result from hybridization. The total number of individuals sequenced is similar to other infraspecific or subspecific studies (Lecointre et al. 1993). Total DNA was extracted from muscle tissue. A 623 base pair (bp) section of the mtDNA genome from the 16s rRNA region was amplified using standard polymerase chain reaction (PCR) procedures. For 25% of the taxa, two or three

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Table 3. Summary table of multiple comparisons after an unbiased estimate of the exact Fisher test (in Genepop 3) for allelic differentiation between samples of *L. soufia*.

	PGI-B	*PGI-A*	*sAAT-A*	*sAAT-B*	*LDH*	*PGM*	*MPI*	*AK-A*	*mIDHP-B*	*G6PDH*	*GP-2*	*ME-2*
All samples	***	--	***	***	**	--	***	--	--	--	***	***
Five samples from Durance River	--	--	--	*	--	--	--	--	--	--	--	--
Seven samples from Rhône Basin	--	--	***	***	**	--	--	--	--	--	--	--
Var River – Rhône Basin	***	--	***	***	*	--	--	--	--	--	--	--
Bevera River – Rhône Basin	***	--	***	***	***	--	***	--	--	--	***	***
Var River – Bevera River – Ardèche River	***	--	***	***	--	--	***	--	--	--	***	***

Note: The nine samples were first compared pairwise for each allozyme locus studied and then, for simplicity, regrouped into sets of comparisons yielding identical results. See Table 1 for different population sample names. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; --, not significant.

different PCRs were carried out on the same specimen and the product was sequenced. In these controls, divergence of less than 0.12% was recorded. Two universal primers were used: 5′-CGCTTGTTTATCAAAAACATGT-3′ (forward) and 5′-CCGCTTTGAGCTCAGATCA-3′ (reverse) (Kocher et al. 1989). Thermal cycle amplifications were performed in 50-µL reaction volumes containing one unit of DNA polymerase (Bioprobe®), 5 µL of reaction buffer (Bioprobe®), 0.4 µL of dNTP (100 mM), and 2.5 µL of each primer (10 µM). One microlitre of the DNA preparation was added to the PCR mix. Cycle parameters were 1 min at 93°C (one cycle); 15 s at 93°C, 45 s at 48°C, and 2 min and 30 s at 72°C (five cycles); 15 s at 93°C, 45 s at 55°C, and 2 min and 30 s at 72°C (30 cycles); and 7 min at 72°C (one cycle). Using the single-stranded DNA as a template, the nucleotide sequence was determined with an automated DNA sequencer (Genome Express®). The DNA sequences were aligned using the MUST (Philippe 1993) and CLUSTAL V (Higgins et al. 1992) programs and the aligned sequences analysed using COMP_MAT programs of MUST and the PHYLIP and PAUP© packages (Swofford 1993).

Results

Morphology

The independence of the different variables was tested by means of the Spearman rank correlation coefficient. The maximum value (r_s = 0.1956) was obtained for the pair of variables P.T. 1 and P.T. 2. The variables ANA and DOR, which are often correlated, exhibited an r_s = 0.007 and a probability p = 0.909 of being independent. The Kruskal–Wallis test (p <0.05) revealed no difference in the number of caudal fin rays for the whole set of nine samples. For the other variables, for which the Kruskal–Wallis test revealed heterogeneity over the whole set of samples, we made multiple comparisons of mean rank. Only the variables PEC, ANA, and GR showed significant differences between the samples (Table 2). For variable ANA, this heterogeneity is due to the differences between the Bevera River sample and all the others. This is indicative of the distinct position of *multicellus*, which is confirmed by analysis of the phenograms based on the bootstrapped meristic data sets using Cavalli-Sforza and Edwards' distance and the UPGMA clustering algorithm (Fig. 2*a*). In its position, *multicellus* was separate in 65 of 100 bootstrapped data sets whereas the other *soufia* is contained within the samples from *agassizi*.

Allozymes

Of the 32 loci scored in *L. soufia* and *A. bipunctatus*, 17 were variable (Appendix 1). Twelve loci were variable for the various samples of *L. soufia*. At seven of these polymorphic loci (*GPI-B*, *sAAT-A*, *sAAT-B*, *LDH-A*, *MPI*, *GP-2*, and *MEP-2*), the samples of *L. soufia* showed significant differences in their allele frequencies (Table 3). *Leuciscus soufia multicellus* from the Bevera River was fixed for three alleles at loci *MPI*, *ME-2*, and *GP-2* and showed clear differences in the frequency of the most common allele at three further loci (*GPI-B*, *sAAT-A*, and *sAAT-B*). *Leuciscus soufia soufia* from the Var River differed from all other samples in the most common allele at locus *GPI-B* but was generally closer in frequency to *multicellus* for *sAAT-A* and *sAAT-B*. The heterozygosities observed were comparable with those recorded for American and European cyprinids (Alves and Coelho 1994). The samples from *agassizi* were the most polymorphic and yielded heterozygosity estimates ranging from 0.037 to 0.063 (Table 4). In *L. s. soufia*, the estimated level of heterozygosity was low (H = 0.016) and was even lower in *multicellus* (H = 0.008).

Results of F statistics calculated by the two methods were very similar (Table 5). The F_{it} value of the samples from the Rhône Basin samples (0.0560) indicates a set of homogenous populations. The Var was very close to this set (F_{it} = 0.1010). However, the average F_{is} value (0.0150) for the different samples from the Rhône Basin samples was low and argues for an overall within-sample Hardy–Weinberg equilibrium. This phenomenon affects all the samples of populations of the various subspecies taken separately (mean F_{is} value = 0.008). The F_{st} value for the Rhône Basin population samples (0.0420) indicates a low degree of genetic differentiation, despite fragmentation of the range. The slightly increased F_{st} value for the Var – Rhône Basin populations (0.091) is indicative of increased genetic heterogeneity of these two basins. The multiple comparison of the F_{st} values for all samples indicates an extensive differentiation between the Bevera sample and the rest, as well as between the Var sample and the rest (Table 6). However, the F_{st} values separating the Bevera sample were very high (mean = 0.8118), which argues for a very strong degree of differentiation between the two basins (Table 6). In addition, we have tested linkage disequilibrium for each pair of loci in every

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Table 4. Measures of genetic variability calculated for nine samples of *L. soufia*.

Population	Subspecies	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic[a]	Mean heterozygosity: Direct count	Mean heterozygosity: Hardy–Weinberg expected[b]
1. Manosque	*L. s. agassizi*	24	1.2	18.8	0.051	0.053
		(0.0)	(0.1)		(0.024)	(0.025)
2. Sisteron	*L. s. agassizi*	28	1.2	21.9	0.042	0.052
		(0.0)	(0.1)		(0.020)	(0.025)
3. Serre-Ponçon	*L. s. agassizi*	30	1.2	18.8	0.063	0.053
		(0.0)	(0.1)		(0.031)	(0.026)
4. Laragne	*L. s. agassizi*	44	1.3	25	0.050	0.051
		(0.0)	(0.1)		(0.023)	(0.024)
5. Lafaurie	*L. s. agassizi*	32	1.2	18.8	0.053	0.053
		(0.0)	(0.1)		(0.025)	(0.026)
6. Ardèche	*L. s. agassizi*	21	1.2	15.6	0.037	0.043
		(0.0)	(0.1)		(0.019)	(0.022)
7. Var	*L. s. soufia*	36	1.2	15.6	0.016	0.015
		(0.0)	(0.1)		(0.009)	(0.008)
8. Bevera	*L. s. multicellus*	29	1	3.1	0.008	0.007
		(0.0)	(0.0)		(0.008)	(0.007)
9. Eau Salée	*L. s. agassizi*	41	1.2	15.6	0.046	0.047
		(0.0)	(0.1)		(0.025)	(0.025)

Note: Standard errors in parentheses.
[a]A locus is considered polymorphic if more than one allele was detected.
[b]Unbiased estimate (Nei 1978).

Table 5. *F* statistics and standard errors calculated by jackknifing at all loci between the different samples of *L. s. agassizi* and between *L. s. soufia* and *L. s. agassizi*.

	F_{it}	F	F_{st}	θ	F_{is}	f
Five samples from Durance River	0.0180	0.0190±0.0160	0.0170	0.0081±0.0085	0.0000	0.0110±0.0091
Seven samples from Rhône Basin	0.0560	0.0532±0.0323	0.0420	0.0293±0.0156	0.0150	0.0244±0.0207
Var River – Rhône Basin	0.1010	0.1094±0.0255	0.0910	0.0913±0.0155	0.0110	0.0199±0.0176

Note: See Table 1 for the different population sample names.

Table 6. Estimating intraclass correlation.

	Bevera	Var	Eau salée	Manosque	Sisteron	Serre-Ponçon	Laragne	Lafaurie
Var	**0.9141**							
Eau salée	***0.7944***	*0.2970*						
Manosque	***0.7991***	*0.2236*	*0.0230*					
Sisteron	***0.7944***	*0.2330*	*0.0076*	*–0.0137*				
Serre-Ponçon	***0.7976***	*0.2964*	*–0.0062*	*0.0117*	*–0.0019*			
Laragne	***0.7553***	*0.1972*	*0.0446*	*0.0034*	*0.0145*	*0.0486*		
Lafaurie	***0.7796***	*0.2393*	*0.0078*	*–0.0118*	*–0.0158*	*0.0011*	*0.0109*	
Ardèche	***0.8603***	*0.4864*	*0.0414*	*0.1098*	*0.0827*	*0.0292*	*0.1690*	*0.0837*

Note: The information considered is allele identity (F_{st}, following Weir and Cockerham 1994 as implemented in Genepop 3) for all population pairs. See Table 1 for the different population sample names. Bold data: comparison with the subspecies *multicellus*; italic data: comparison with the subspecies *soufia*; underlined data: comparison with the subspecies *agassizi*.

population sampled. Only the Lafaurie population representing *agassizi* exhibited significant linkage disequilibrium for the locus pair *sAAT-A* and *sAAT-B*. Despite this, the panmixia of samples of *agassizi* can be assumed.

A UPGMA dendrogram based on Nei's genetic distance was constructed (Fig. 2*b*). Apart from the Ardèche, which exhibited slight differentiation (due to the inversion of the allelic frequencies at loci *sAAT-A* and *sAAT-B*), all the other samples belonging to *agassizi* collapsed into a single group. The Var River is slightly differentiated from this set because of its monomorphism (absence of rare and (or) specific alleles). Finally, the Bevera River is differentiated from this set by allele *A* at locus *MPI* and by allele *B* at loci *GP-2* and *ME-2*, as well as by extreme monomorphism. Another phenogram, based on Cavalli-Sforza and Edwards' distance using the UPGMA clustering algorithm on 100 bootstrapped data sets, also indicated high genetic differentiation of the sample from the Bevera and the Var (Fig. 2*c*). In 98 and 90

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Table 7. mtDNA halotypes for 18 taxa including *C. carpio* (Chang et al. 1994) and two *A. bipunctatus* as outgroups, variable nucleotide positions, numbers of individuals sampled (*N*), and frequency distribution of halotypes among the total data set.

						Locality No. (*N*)											
						1	2	3	4	5	6	7	8	9	10	11	Total
						(12)	(9)	(9)	(10)	(9)	(10)	(17)	(12)	(10)	(4)	(4)	(106)
		1111	2222222222	2223333333	3333334445	55											
		1110579	3456666788	8992234455	5567771290	00											
		3890129479	8421356246	7036814902	7871492200	69											
C. carpio		AAACCAAUCA	AAAAUAAAAC	AAUGAGCAAA	CUGGAUGAGG	GG											
A. bipunctatus(1)		-C-UUG-CUU	G--U--G-CA	--CAGCUCC-	-*---C-G-A	--										1	1
A. bipunctatus(2)		-C-UUG-CUU	G--U--G-CA	--CAG-UCC-	-*---C-G--	--										3	3
Lin 1	Hap 1	CU-UUGG--U	---CCG-CCG	---AU---UG	--UU--A---	C-									1		1
Lin 1	Hap 2	CU-UUGG--U	---CCG-CCG	---AU---UG	--UU--A---	--									3		3
Lin 1	Hap 3	CU-UUGG--U	-G-CCG-CCG	---AU---UG	--UU--A-A-	--						1	8				9
Lin 1	Hap 4	CU-UUGG--U	-G-CCG-CCG	---AU---UG	--UU--A-A-	C-						1					1
Lin 2	Hap 5	--U-**----	G--G-G-CUA	CG--GU-CC-	U*UUG-A-A-	--						1					1
Lin 2	Hap 6	--U-**----	G--G-G-CUA	CG--GU-CC-	U*UUG-A-A-	--	9	7	8	7	6	9	13		9			68
Lin 2	Hap 7	--U-**----	G--G-G-CUA	CG--GU-CC-	U*UUG-A-A-	C-				1			1	4				6
Lin 2	Hap 8	--U-**----	G--G-G-CUA	CG--GU-CC-	U*UUG-A-AA	--	1											1
Lin 2	Hap 9	--U-**----	G--G-G-CUA	CG--GU-CC-	U*UUG-A-A-	-U		1										1
Lin 2	Hap 10	--U-**----	G-GG-G-CUA	CG--GU-CC-	U*UUG-A-A-	--		1										1
Lin 2	Hap 11	--U-**----	G--G-G-CUA	CG--GU-CC-	U*UUG-A---	-U				1								1
Lin 2	Hap 12	--U-**----	G--G-G-CUA	CG--GU-CC-	U*UUG-A---	--					1							1
Lin 2	Hap 13	--U-**----	G--G-G-CUA	CG--GU-CC-	U*UUG-A---	--	2			1	2							5
Lin 2	Hap 14	--U-**----	G-UG-G-CUA	CG--GU-CC-	U*UUG-A-A-	C-			1									1
Lin 2	Hap 15	--U-**----	G-GG-G-CUA	CG--GU-CC-	U*UUG-A---	--					1			1				2

Note: Asterisks represent a gap.

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of the 100 bootstrapped data sets, *multicellus* and *soufia* formed separate clusters, respectively. Within *agassizi*, the sample from the Ardèche branched first, leaving the others in a close cluster.

mtDNA

A 582-bp DNA fragment was aligned for 18 taxa, including *Cyprinus carpio* (Chang et al. 1994) and two *A. bipunctatus* as outgroups (Appendix 2). The analysis was carried out on 549 bp, while 33 bp were excluded because of the lack of sequence for some individuals. This database showed no sign of saturation and provided 87 variable sites and 42 informative sites for parsimony analysis. The distance matrix showed that the subfamily Cyprininae, represented by *C. carpio*, is distant from the subfamilies Leucicinae and Alburninae. In addition, the subfamily Alburninae, represented in this study by *A. bipunctatus*, is very closely allied to *L. soufia*. We detected two *L. soufia* lineages, representing 15 different haplotypes (Hap 1–15). Among these haplotypes, 30 variable sites were detected, 29 of which were informative for parsimony analysis (Table 7). Four of these haplotypes (Hap 1–4) were exclusive to the *multicellus* lineage (Lin 1), exhibiting three variable and informative sites among them. Eleven haplotypes (Hap 5–15) belonged to the *agassizi* lineage (Lin 2), exhibiting five variable sites that included four informative sites.

Bootstrap analyses with 1000 iterations using parsimony (PAUP) and neighbour-joining methods (MUST) yielded identical tree topologies. The branch lengths were also similar (10–17 and 8–15), indicating a constant rate of evolution in the two lineages. The first mtDNA lineage (Hap 5–15) included all the specimens of *agassizi*, the 15 specimens from the Var (*soufia*), and four specimens from the Bevera (*multicellus*). The second mtDNA lineage (Hap 1–4) included eight specimens from the Bevera, four specimens from the Pô (*multicellus*), and two specimens from the Var (*soufia*). However, within the two lineages, low levels of inter- and intra-populational sequence variability were detected, with the notable exception of Hap 3 and 4 in Lin 1, representing the samples from the River Pô, which appear to be well differentiated (Fig. 2*d*).

Discussion

The currently accepted taxonomy in *L. soufia* includes the three morphologically distinctive subspecies *L. s. soufia*, *L. s. agassizi*, and *L. s. multicellus*. Our data show that the samples from the Rhône Basin, representing *agassizi*, exhibit no significant enzymatic or molecular differentiation. The rare alleles diagnosed by enzymatic analysis may originate from intraspecific enzyme polymorphism or occasional introgression of females of *L. soufia* with other cyprinids. A second taxonomic entity is the sample from the Bevera River, representing *multicellus*, which morphologically as well as genetically is the most highly differentiated of the group. Lynch and Jarrell (1993) showed that in ectotherms, the different regions of the mtDNA do not evolve at the same rate. Therefore, in order to estimate divergence times between the lineages, we have used, for the 16s rDNA, a rate of evolution of 0.7% nucleotide divergence per million years that was calibrated by the studies of Miya and Nishida (1996) and Gilles et al. (1998). Avise (1994) estimated the percentage of divergence between two lineage on the basis of the relationship $d = Z_d/Z_t$ (where Z_d is the number of nucleotides that differ between two sequences and Z_t is the total number of nucleotides compared). In the case of *agassizi* and *multicellus*, we obtained $4.60\% < d < 5.34\%$, which provided an estimate for the time of initial separation for the two lineages at about 6–8 million years ago.

With regard to the mtDNA data, four specimens from the Bevera River had mtDNA very similar to that of *agassizi* from the Rhône; the other eight specimens had mtDNA similar to that of the Pô Basin specimens of *multicellus*. It seems evident that *multicellus* exhibits a high rate of introgressed individuals, which is evidence of the close relationships that have developed between this subspecies and *agassizi*. The third taxonomic entity, *L. s. soufia* represented by the sample from the Var River, has not attained a level of genetic differentiation strong enough for attributing subspecies rank. It must rather be considered an isolated population of *agassizi* in the process of differentiation. However, on the basis of morphological characteristics and allozyme data, it belongs to *agassizi*. On the other hand, on the basis of the mtDNA data, it has affinities with both *multicellus* and *agassizi*, as two specimens have a haplotype very similar to that from Bevera River (*multicellus*) and the other 15 specimens have an mtDNA haplotype very close to that of the Rhône Basin (*agassizi*). We therefore think that the Var population was founded by *agassizi* from the Rhône Basin, which was later introgressed by *multicellus* from the Bevera River region in a contact area of the alpine border. In addition, the data suggest that the introgression took place during the Pleistocene rather than recently. This is because the four introgressed specimens caught in the Bevera River bear an mtDNA haplotype of *agassizi* that is very rare in the Rhône Basin whereas if it were a case of recent introgression, the most frequent haplotype should be found. In addition, the combination of a high level of mitochondrial polymorphism and a low level of nuclear polymorphism in the samples from the Var and the Bevera rivers further suggests an ancient process of introgression (Brito et al. 1997). The finding of a linkage disequilibrium would have further supported this hypothesis. These results explain the difficulties encountered by the various authors in attributing and validating a taxonomic rank to these different population samples. The hypothesis that the samples studied form one single population and that the patterns observed can be explained by coalescence should now be dismissed. In fact, the very high degree of divergence between the two lineages (*multicellus* and *agassizi*) place the initial separation between 6 and 8 million years ago. If the populations of the Var and Bevera rivers (that we consider as introgressed, since both haplotypes are present) had resulted from this initial fragmentation, one of the two haplotypes would have disappeared by a simple stochastic process in the course of the 6–8 million years that have elapsed since the separation of the two populations. According to Avise (1994), "In general, it is unlikely that two or more founding lineages by chance will survive beyond $4N$ generations, where N is the population size."

It would appear that *L. soufia* has differentiated into two entities in allopatry in the Messinian caused by the separation of river basins due to the changing sea level (Hsü et al.

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1977), similar to the origin of diversity in mussels (Quesada et al. 1995) and North America freshwater fishes (Todd and Hatcher 1993). A second drop in sea level during the Pleistocene glaciation maxima (Thunell and Williams 1983) enabled secondary contact and restricted introgressive hybridization between the isolated population groups of the Var and Bevera River regions by passing through the brackish or even freshwater parts of the Mediterranean. The subsequent rise in sea level created the present distribution patterns.

With regard to origin of colonization of the present-day distribution, palaeohistoric processes must have played a crucial role. The fossils discovered in the Oligocene deposits of Limagnes, which have been attributed to the genus *Paleoleuciscus* (Gaudant 1984), suggest an old colonization of southern France by cyprinids. Considerable controversy surrounds the colonization and the formation of many endemic forms of cyprinid fishes in southern Europe. Spillmann (1961), Banarescu (1973), and Almaça (1976) argued that they originated from fish populations passing from the north through river connections that occurred between central and southern peninsular European basins in the Miocene or even earlier whereas Bianco (1990) proposed that colonization from the south was more plausible. As in a *Salmo salar* study (Cutler et al. 1991), the 16s rRNA region was a good genetic marker, although whether the ancestral form of *L. soufia* originated in the north or in the south cannot be answered at this stage.

The present study reveals two geographically structured entities of *L. soufia*, representative of the present-day hydrogeographic basins, and entails a revision of the classical system by classifying *L. s. agassizi* and *L. s. soufia* together as *L. s. soufia* while keeping *L. s. multicellus* separate. In addition, the subspecies level presents a real problem because the genus *Leuciscus* is paraphyletic (Gilles et al. 1998). It would be preferable to regroup these two taxonomic entities in their former genus *Telestes*. Furthermore, we argue for two processes of isolation and colonization via the Mediterranean region at different times and introgression, rather than a static process of isolation through the formation of the Alpine range as Banarescu and Almaça have suggested (in Bianco 1990). In addition, *L. soufia* is considered an endangered species in Switzerland, and its distribution has been much reduced as the result of human pressure in Italy and France. Following Ryder's approach (in Avise and Hamrick 1996) based on the evolutionarily significant unit, this study argues for the protection of both subspecies as separate entities (Lande 1988).

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References

Almaça, C. 1976. La spéciation chez les cyprinidae de la péninsule ibérique. Rev. Trav. Inst. Pêches Marit. **40**: 399–411.

Alves, M., and Coelho, M. 1994. Genetic variation and population subdivision of the endangered Iberian cyprinid *Chondrostoma lusitanicum*. J. Fish Biol. **44**: 627–636.

Avise, J. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, New York.

Avise, J., and Hamrick, J. 1996. Conservation genetics. Chapman and Hall, New York.

Banarescu, P. 1973. Some reconsiderations on the zoogeography of the euro-mediterranean fresh-water fish fauna. Rev. Roum. Zool. **18**: 257–264.

Bernatchez, L., Glémet, H., Wilson, C., and Danzmann, R. 1995. Introgression and fixation of Artic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. **52**: 179–185.

Bianco, P. 1990. Potential role of the palaeohistory of the Mediterranean and Paratethys basins on the early dispersal of euro-mediterranean freshwater fishes. Ichthyol. Explor. Freshwaters, **1**: 167–184.

Brito, M., Briolay, J., Galtier, N., Bouvet Y., and Coehlo M. 1997. Phylogenetic relationships within genus *Leuciscus* (Pisces, Cyprinidae) in Portuguese fresh waters, based on mitochondrial DNA cytochrome *b* sequences. Mol. Phylogenet. Evol. **8**: 435–442.

Chang, Y., Huang, F., and Lo, T. 1994. The complete nucleotide sequence and gene organisation of carp (*Cyprinus carpio*) mitochondrial genome. J. Mol. Evol. **38**: 138–155.

Chappaz, R., and Brun, G. 1993. Données nouvelles sur la biologie et l'écologie d'un poisson Cyprinidé du Sud de l'Europe *Leuciscus (Telestes) soufia* (Risso, 1826). C.R. Acad. Sci. Sér. III Sci. Vie, **316**: 35–41.

Cutler, M., Bartlett, S., Hartley, S., and Davidson, W. 1991. A polymorphism in the ribosomal RNA genes distinguishes Atlantic salmon, *Salmo salar* L., from America and Europe. Can. J. Fish. Aquat. Sci. **48**: 1655–1661.

Darlington, P. 1957. Zoogeography: the geographical distribution of animals. Wiley, London, U.K.

d'Aubenton, F., Daget, J., and Spillmann, J. 1971. Classification numérique des blageons *Leuciscus (Telestes) soufia* (Pisces, Cyprinidae). Bull. Mus. Natl. Hist. Nat. **42**: 839–848.

Dobzhansky, T. 1951. Genetics and the origin of species. 3th ed. Columbia University Press, New York.

Dowling, T., and DeMarais, B. 1993. Evolutionary significance of introgressive hybridization in cyprinid fishes. Nature (Lond.), **362**: 444–446.

Dowling, T., and Secor, C. 1997. The role of hybridization and introgression in the diversification of animals. Annu. Rev. Ecol. Syst. **28**: 593–619.

Felsenstein, J. 1989. PHYLIP — phylogeny inference package. Version 3.2. Cladistics, **5**: 164–166.

Gaudant, J. 1984. Nouvelles recherches sur les Cyprinidae (Poisson Téléostéens) dans l'oligocène des limagnes. Géobios (Lyon), **17**. 659–666.

Gilles, A., Barascud, B., Bouchard, P., and Chappaz, R. 1996. Etude de la variabilité de *Leuciscus soufia*, poisson cyprinidé, par analyse du polymorphisme enzymatique et de caractères méristiques. C.R. Acad. Sci. Sér. III Sci. Vie, **319**: 393–399.

Gilles, A., Lecointre, G., Faure, E., Chappaz, R., and Brun, G. 1998. Mitochondrial phylogeny of the European cyprinids: implications for their systematics, reticulate evolution and colonization time. Mol. Phylogenet. Evol. **10**: 132–143.

Higgins, D., Bleasby, A., and Fuchs, R. 1992. CLUSTAL V: improved software for multiple sequence alignment. Comput. Appl. Biosci. **8**: 189–191.

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Hsü, K., Montardet, L., Bernouilli, D., Cita, M., Erickson, A., Garrison, R., Kidd, R., Melietes, F., Müller, C., and Wright, R. 1977. History of the Mediterranean salinity crisis. Nature (Lond.), **267**: 399–403.

Kocher, T.,Thomas, W., Meyer, A., Edwards, S., Pääbo, S., Villablanca, F., and Wilson, A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. U.S.A. **86**: 6196–6200.

Lande, R. 1988. Genetics and demography in biological conservation. Science (Washington, D.C.), **241**: 1455–1460.

Lecointre, G., Philippe, H., Lê, H., and Le Guyader, H. 1993. Species sampling has a major impact on phylogeny inference. Mol. Phylogenet. Evol. **2**: 205–224.

Lydeard, C., Wooten, M., and Meyer, A. 1995. Molecules, morphology, and area cladograms: a cladistics and biogeographic analysis of *Gambusia* (Teleostei: Poeciliidae). Syst. Zool. **44**: 221–234.

Lynch, M., and Jarrell, E. 1993. A method for calibrating molecular clocks and its application to animal mitochondrial DNA. Genetics, **135**: 1197–1208.

Mayr, E. 1963. Animal species and evolution. Belknap. Harvard University Press, Cambridge, Mass.

Miya, M., and Nishida, M. 1996. Molecular phylogenetic perspective on the evolution of the deep-sea fish genus *Cyclothone* (Stomiiformes: Gonostomatidae). Ichthyol. Res. **43**: 375–398.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance for a small number of individuals. Genetics, **89**: 583–590.

Philippe, H., 1993. MUST: a computer package of management utilities for sequences and trees. Nucleic Acids Research. **21**: 5264–5272.

Quesada, H., Wenne, R., and Skibinski., D. 1995. Differential introgression of mitochondrial DNA across species boundaries within the marine mussel genus *Mytilus*. Proc. R. Soc. Lond. Ser. B Biol. Sci. **262**: 51–56.

Raymond, M., and Rousset, F. 1995. Genepop (version 3): population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249.

Shaklee, J., Allendorf, F., Morizot, D., and Whitt, G. 1990. Gene nomenclature for protein-coding loci in fish. Trans. Am. Fish. Soc. **119**: 2–15.

Spillmann, J. 1961. Faune de France. Vol. 61. Paul Lechevalier, Paris, France. Paris.

Sturmbaeur, C., and Meyer, A. 1992. Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. Nature (Lond.), **358**: 578–581.

Swofford, D.L. 1993. Phylogenetic analysis using parsimony (PAUP) 3.1.1. Illinois Natural History Survey, Champaign, Ill.

Swofford, D., and Selander, R. 1981. Biosys-1: a Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. **72**: 281–283.

Thunell, R., and Williams, D. 1983. Paleotemperature and paleosalinity history of the eastern Mediterranean during the Late Quaternary. Palaeogeogr. Palaeoclimatol. Palaeoecol. **44**: 23–39.

Todd, T., and Hatcher, C. 1993. Genetic variability and glacial origins of the yellow perch (*Perca flavescens*) in North America. Can. J. Fish. Aquat. Sci. **50**: 1828–1834.

Tomassone, R., Dervin, C., and Masson, J.P. 1993. Biométrie, modélisation de phénomènes biologiques. Masson, Paris, France.

Ward, R., and Elliott, N. 1993. Heterozygosity and morphological variability in the orange roughy, *Hoplostethus atlanticus* (Teleostei: Trachichthyidae). Can. J. Fish. Aquat. Sci. **50**: 1641–1649.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Wynne, I., Loxdale, H., and Brookes, C. 1992. Use of a cellulose acetate system for allozyme electophoresis. *In* Genes in ecology. *Edited by* R.J. Berry, T.J. Crawford, and G.M. Hewitt. Blackwell, Oxford, U.K. pp. 494–499.

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Appendix 1. Allele frequencies for 17 of the 32 loci scored.

	Population (*N*)									
Locus	1 (24)	2 (28)	3 (30)	4 (44)	5 (32)	6 (21)	7 (36)	8 (29)	9 (41)	Outgroup (14)
GPI-B										
A	0.625	0.643	0.667	0.545	0.609	0.738	0.986	0.121	0.646	1.000
B	0.375	0.357	0.333	0.455	0.391	0.262	0.014	0.879	0.354	0.000
GPI-A										
A	0.979	0.946	0.950	0.989	0.969	1.000	0.986	1.000	0.988	0.000
B	0.000	0.054	0.050	0.011	0.031	0.000	0.014	0.000	0.012	0.000
C	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
sAAT-A										
A	0.333	0.375	0.517	0.341	0.391	0.714	0.083	0.000	0.561	0.000
B	0.667	0.625	0.483	0.659	0.609	0.286	0.917	1.000	0.439	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
sAAT-B										
A	0.583	0.554	0.500	0.739	0.563	0.333	0.889	1.000	0.573	0.000
B	0.417	0.446	0.500	0.261	0.438	0.667	0.111	0.000	0.427	1.000
LDH-A										
A	0.896	0.982	0.967	0.932	0.938	0.976	0.972	1.000	1.000	1.000
B	0.104	0.018	0.033	0.068	0.016	0.024	0.028	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000
PGM										
A	1.000	0.982	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MPI										
A	0.000	0.000	0.000	0.011	0.000	0.000	0.000	1.000	0.000	0.000
B	1.000	0.982	1.000	0.966	0.969	0.976	1.000	0.000	1.000	1.000
C	0.000	0.018	0.000	0.023	0.031	0.024	0.000	0.000	0.000	0.000
AK-A										
A	0.979	1.000	0.967	0.977	1.000	1.000	1.000	1.000	1.000	1.000
B	0.021	0.000	0.033	0.023	0.000	0.000	0.000	0.000	0.000	0.000
mIDHP-B										
A	1.000	1.000	1.000	0.989	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000
G6PDH										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.988	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000
EST-3										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
sSOD										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
sIDDH										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
CK-A										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
FH										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
ME-2										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000
GP-2										
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000

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Appendix 2. Aligned sequences of the 16s rRNA for the different halotypes of *L. soufia* (see Table 7 for their distribution), two *A. bipunctatus*, and one *C. carpio* (Chang et al. 1994).

	1 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777778 8888888889 9999999990									
	1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890									
C. carpio	GCAAC*GAAC	CAAGUAUAGG	AGGUCCAGCC	UGCCCAGUGA	CUACAAGUUC	AACGGCCCGG	CGGUUAUUUU	GACCGUGCAA	AGGUAGCGCA	AUCACUUGUC
A. bipunctatus(1)	-----UAC-U	UG--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
A. bipunctatus(2)	-----UAC-U	UG--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 1 HAP 1	--C--UAU-U	UG--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 1 HAP 2	--C--UAU-U	UG--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 1 HAP 3	--C--UAU-U	UG--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 1 HAP 4	--C--UAU-U	UG--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 5	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 6	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 7	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 8	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 9	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 10	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 11	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 12	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 13	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 14	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------
LIN 2 HAP 15	-----UA-U-	**--------	----------	----------	----GG----	-------**-	---*------	----------	----------	----------

	1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 1111111112									
	0000000001 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777778 8888888889 9999999990									
	1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890									
C. carpio	UUUUAAAUAG	AGACCUGUAU	GAAUGGCUAA	ACGAGGGCUU	AACUGUCUCC	CCUUUCAAGU	CAGUGAAAUU	GAUCUACCCG	UGCAGAAGCG	GGUAUAAUAC
A. bipunctatus(1)	-C---------	----------	---------G	----------	----------	--CC--C---	----------	------U---	----------	--------UA
A. bipunctatus(2)	-C---------	----------	---------G	----------	----------	--CC--C---	----------	------U---	----------	--------UA
LIN 1 HAP 1	-C------G-	----------	---------G	----------	----------	--C---C---	----------	----------	----------	--------UA
LIN 1 HAP 2	-C------G-	----------	---------G	----------	----------	--C---C---	----------	----------	----------	--------UA
LIN 1 HAP 3	-C------G-	----------	---------G	----------	----------	--C---C---	----------	----------	----------	--------UA
LIN 1 HAP 4	-C------G-	----------	---------G	----------	----------	--C---C---	----------	----------	----------	--------UA
LIN 2 HAP 5	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 6	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 7	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 8	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 9	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 10	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 11	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 12	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 13	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 14	-C---------	----------	---------G	--A-------	----------	--C---C---	----------	----------	----------	---------A
LIN 2 HAP 15	-C---------	----------	---------G	----------	----------	--C---C---	----------	----------	----------	---------A

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Appendix 2 (*continued*).

	2222222222	2222222222	2222222222	2222222222	2222222222	2222222222	2222222222	2222222222	2222222222	2222222223
	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	7777777778	8888888889	9999999990
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
C. carpio	UACAAGACGA	GAAGACCCUU	UGGAGCUUAA	GGUACAAAAC	UCAACCACGU	UAAGCAACUC	AAUAAAAAGC	AAAAACCUUG	UGGAUCAUGA	GAUUUUACCU
A. bipunctatus(1)	----------	----------	---------G	-------G-U	----------	---A-G----	U----G----	--G---U-A-	---CGA----	A-C-------
A. bipunctatus(2)	----------	----------	----------	-------G-U	----------	---A-G----	U----G----	--G---U-A-	---CGA----	A-C-------
LIN 1 HAP 1	----------	----------	----------	---------U	----------	---A-G----	C-C-G-----	-CG---U-A-	---CGG----	A---------
LIN 1 HAP 2	----------	----------	----------	---------U	----------	---A-G----	C-C-G-----	-CG---U-A-	---CGG----	A---------
LIN 1 HAP 3	----------	----------	----------	---------U	---G------	---A-G----	C-C-G-----	-CG---U-A-	---CGG----	A---------
LIN 1 HAP 4	----------	----------	----------	---------U	---G------	---A-G----	C-C-G-----	-CG---U-A-	---CGG----	A---------
LIN 2 HAP 5	----------	----------	----------	-------G-U	----------	---A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 6	----------	----------	----------	-------G-U	----------	---A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 7	----------	----------	----------	-------G-U	----------	---A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 8	----------	----------	----------	-------G-U	----------	---A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 9	----------	----------	----------	-------G-U	----------	---A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 10	----------	----------	----------	-------G-U	----------	-G-A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 11	----------	----------	----------	-------G-U	----------	---A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 12	----------	----------	----------	-------G-U	----------	---A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 13	----------	----------	----------	-------G-U	----------	---A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 14	----------	----------	----------	-------G-U	----------	-U-A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------
LIN 2 HAP 15	----------	----------	----------	-------G-U	----------	-G-A-G----	G---G-----	-CG---U-A-	---UGAC--G	A---------

	3333333333	3333333333	3333333333	3333333333	3333333333	3333333333	3333333333	3333333333	3333333333	3333333334
	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	7777777778	8888888889	9999999990
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
C. carpio	UCGGUUGGGG	CGACCACGGA	GGAAAGAAAA	GCCUCCAGGU	GGACUGGGAA	AACCUCCUAA	AACCAAGAGA	GACAUCUCUA	AGC*ACAGAA	CAUCUGACCA
A. bipunctatus(1)	----------	----------	-----A-G--	C-----GA--	---U----CC	--A-C--*--	-G--------	-------AC-	---CG-----	----------
A. bipunctatus(2)	----------	----------	-----A-G--	------GA--	---U----CC	--A-C--*--	-G--------	-------AC-	---CG-----	----------
LIN 1 HAP 1	----------	----------	-----A-U--	------GA--	---------U	-GA-C-----	-G----U---	U------A--	---CG-----	----------
LIN 1 HAP 2	----------	----------	-----A-U--	------GA--	---------U	-GA-C-----	-G----U---	U------A--	---CG-----	----------
LIN 1 HAP 3	----------	----------	-----A-U--	------GA--	---------U	-GA-C-----	-G----U---	U------A--	---CG-----	----------
LIN 1 HAP 4	----------	----------	-----A-U--	------GA--	---------U	-GA-C-----	-G----U---	U------A--	---CG-----	----------
LIN 2 HAP 5	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 6	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 7	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 8	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 9	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 10	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 11	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 12	----------	----------	-------GG-	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 13	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 14	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------
LIN 2 HAP 15	----------	----------	-------G--	U-----GA--	--------CC	--A-C-U*--	-G----U---	U--G---A--	---CG-----	----------

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Appendix 2 (*concluded*).

	4444444444	4444444444	4444444444	4444444444	4444444444	4444444444	4444444444	4444444444	4444444444	4444444445
	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	6666666667	7777777778	8888888889	9999999990
	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890
C. carpio	AAUAUGAUCC	GGCUAACACA	UAGCCGAUCA	ACGAACCAAG	UUACCCUAGG	GAUAACAGCG	CAAUCCUCUC	CCAGAGUCCA	UAUCGACGAG	GGGGUUUACG
A. bipunctatus(1)	--A-------	----G-A-**	*G--------	--------UU	----------	----------	----------	---A------	-U-----A--	---------A
A. bipunctatus(2)	--A-------	----G-A-**	*G--------	----------	----------	----------	----------	----------	----------	----------
LIN 1 HAP 1	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	----------	----------
LIN 1 HAP 2	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	----------	----------
LIN 1 HAP 3	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	---------A	----------
LIN 1 HAP 4	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	---------A	----------
LIN 2 HAP 5	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	---------A	----------
LIN 2 HAP 6	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	---------A	----------
LIN 2 HAP 7	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	---------A	----------
LIN 2 HAP 8	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	---------A	---------A
LIN 2 HAP 9	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	---------A	----------
LIN 2 HAP 10	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	---------A	----------
LIN 2 HAP 11	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	----------	----------
LIN 2 HAP 12	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	----------	----------
LIN 2 HAP 13	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	----------	----------
LIN 2 HAP 14	--A-------	-A--G-A-**	*---------	----------	--U-------	----------	----------	----------	---------A	----------
LIN 2 HAP 15	--A-------	-A--G-A-**	*---------	----------	----------	----------	----------	----------	----------	----------

	5555555555	5555555555	5555555555	5555555555	555555555
	0000000001	1111111112	2222222223	3333333334	444444444
	1234567890	1234567890	1234567890	1234567890	123456789
C. carpio	ACCUCGAUGU	UGGAUCAGGA	CAUCCUAAUG	GUGCAGCCGC	UAUUAAGGG
A. bipunctatus(1)	----------	----------	----------	----------	---------
A. bipunctatus(2)	----------	----------	----------	----------	---------
LIN 1 HAP 1	-----C----	----------	----------	----------	---------
LIN 1 HAP 2	----------	----------	----------	----------	---------
LIN 1 HAP 3	----------	----------	----------	----------	---------
LIN 1 HAP 4	-----C----	----------	----------	----------	---------
LIN 2 HAP 5	-A--------	----------	----------	----------	---------
LIN 2 HAP 6	----------	----------	----------	----------	---------
LIN 2 HAP 7	-----C----	-U--------	----------	----------	---------
LIN 2 HAP 8	----------	----------	----------	----------	---------
LIN 2 HAP 9	--------U-	----------	----------	----------	---------
LIN 2 HAP 10	----------	----------	----------	----------	---------
LIN 2 HAP 11	--------U-	----------	----------	----------	---------
LIN 2 HAP 12	----------	----------	----------	----------	---------
LIN 2 HAP 13	----------	----------	----------	----------	---------
LIN 2 HAP 14	-----C----	----------	----------	----------	---------
LIN 2 HAP 15	----------	----------	----------	----------	---------

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PERSPECTIVE

Simple dynamics underlie sockeye salmon (*Oncorhynchus nerka*) cycles

Ransom A. Myers, Gordon Mertz, Jessica M. Bridson, and Michael J. Bradford

Abstract: A variety of mechanisms have been proposed to explain the renowned British Columbia sockeye salmon (*Oncorhynchus nerka*) cycles, most of which invoke between-brood interactions (delayed density dependence) or depensatory harvest practices. We examine the dynamics of the Ricker model with realistic parameter values and suggest that the cycles could result from a stable mode excited by stochastic forcing. The previously proposed mechanisms are not required to generate cyclic patterns, although they could play a role in reinforcing cycles. Our results suggest that relaxing harvest rates will increase yields and decrease variability in sockeye abundances.

Résumé : On a envisagé toutes sortes de mécanismes pour expliquer les cycles bien connus qu'on observe chez le saumon rouge (*Oncorhynchus nerka*) de la Colombie-Britannique : dans la plupart des cas, on suppose des interactions entre les pontes annuelles (effet retardé dépendant de la densité) ou des pratiques de pêche anticompensatoires. Nous avons étudié la dynamique du modèle de Ricker en attribuant aux paramètres des valeurs réalistes et nous pensons que les cycles du saumon rouge pourraient refléter un mode stable stimulé par un forçage stochastique. Les mécanismes envisagés antérieurement ne devaient pas nécessairement provoquer un effet cyclique, même s'ils pouvaient avoir un effet de renforcement. D'après les résultats que nous avons obtenus, la diminution de la pêche devrait se traduire par une augmentation des rendements et une baisse de la variabilité des effectifs du saumon rouge.

[Traduit par la Rédaction]

Introduction

The sockeye salmon (*Oncorhynchus nerka*) stocks of British Columbia provide perhaps the clearest example of cycling fish populations. For a number of stocks the abundance of returning spawners exhibits an oscillation of remarkable amplitude and regularity (Fig. 1) at a period of 4 years, equal to the age at maturity. The challenge posed to population dynamicists is evident (Ricker 1950; see Levy and Wood 1992 and Ricker 1997 for a review of mechanisms proposed to explain these cycles).

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e-mail: ransom.myers@dal.ca
[2]My good friend Gordon Mertz tragically died after this manuscript was finalized; his intelligence, humor, and charm will be greatly missed — R.A. Myers.

Most models of these cycles have emphasized delayed density-dependent mortality, in which a strong year-class suppresses one or more year-classes following it (Larkin 1971; Collie and Walters 1987; Walters and Staley 1987). It can be shown through simulation (Larkin 1971; Collie and Walters 1987; Walters and Staley 1987) that models incorporating delayed density dependence can produce analogs of the sockeye cycles. However, to date, it has not been demonstrated that delayed density dependence is necessary to generate sockeye-like cycles. Furthermore, in the metaanalysis carried out by Myers et al. (1997), we were unable to detect any more than a weak delayed density-dependent mortality at a 1-year lag and no convincing evidence at greater lags. In this paper, we will show that the sockeye cycles can exist independent of delayed density dependence and other proposed influences such as genetic factors (Walters and Woodey 1992) or depensatory fishing, i.e., fishing mortality that is inversely related to abundance (Peterman 1980; Ricker 1987). We demonstrate that a strikingly simple mechanism appears to underly the cycles, namely the stochastic forcing of a previously unrecognized stable mode.

Theory

Our analysis will commence with an examination of a very simple model, which will be shown to be capable of generating sockeye-like cycles without any delayed density dependence or depensatory fishing whatsoever.

We adopt Ricker's (1954) framework for the salmon popu-

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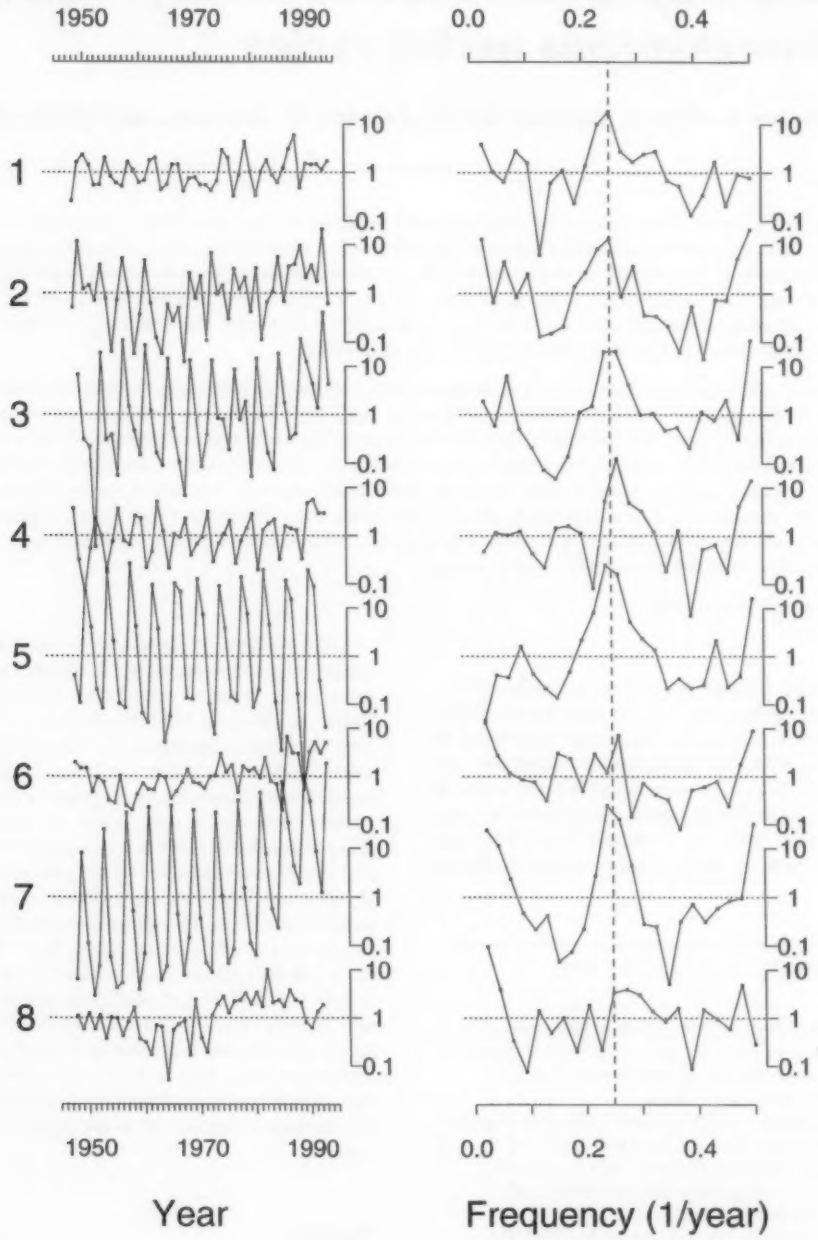
Fig. 1. Time-series (left) and spectra (right) of the log number of female spawners for the eight major populations in the Fraser River: 1, Stellako River; 2, early run of Stuart watershed; 3, late run of Stuart watershed; 4, Chilko River; 5, Adams River; 6, Birkenhead River; 7, Horsefly River; 8, Weaver Creek. The mean of each series is separated by 2.5 units, i.e., a factor of $10^{2.5} \approx 316.2$, from the one below. Thus the distance between the horizontal broken lines gives the vertical scale. Spectra are raw periodograms with a standard split cosine bell taper of 5% of the values on each end. The broken vertical lines on the periodogram represent the age at maturity (4 years). These data were compiled by the Pacific Salmon Commission.



lation dynamics, and thus the abundance of spawners (S'_t) is governed by

$$(1) \quad S'_{t+4} = \alpha_* S'_t e^{-\beta S'_t + \varepsilon_t}.$$

Here, α_* is the reproductive parameter that is reduced from its natural value (α) by a factor of $1 - H$ where H is the harvest rate ($\alpha_* = (1 - H)\alpha$). (At low population sizes, each spawner produces α_* spawners 4 years later.) The parameter β repre-

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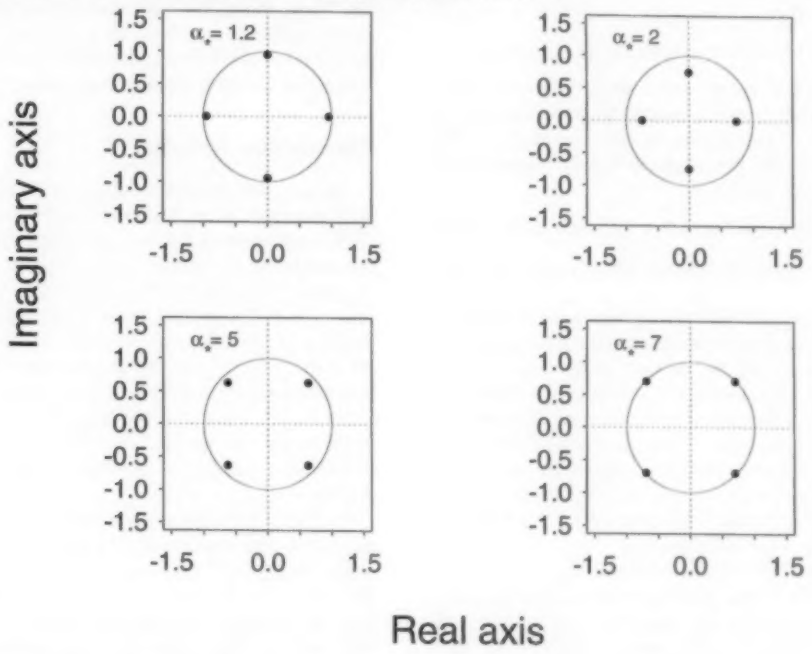
Fig. 2. Complex roots of eq. 2, representing the linearized dynamics, for four different values of α_*, the reproductive parameter. The roots-on-axes pattern prevails for $\alpha_* < e$, becoming a roots-on-diagonals pattern for $\alpha_* > e$.



sents direct (not delayed) density dependence. The variable ε_t is a zero-mean Gaussian process representing the influence of environmental variability on survival from egg to harvestable age. We are neglecting the 5%, on average, of the British Columbia sockeye that spawn at age 5; the proportion is larger for the Birkenhead population, which, interestingly, does not cycle.

The parameter β can be removed from the analysis by forming a new scaled variable S_t, such that $S_t = \beta S'_t$. The population dynamics now read

$$(2) \quad S_{t+4} = \alpha_* S_t e^{-S_t + \varepsilon_t}.$$

It is apparent from eq. 2 that only two input parameters are required, α_* and σ_ε, the standard deviation of the white noise forcing.

Although it might appear that a 4-year cycle follows trivially from eq. 2, the spontaneous oscillations (those arising from instability) have a period of 8 years, a well-known result discovered by Ricker (1954).

Consider the case $\sigma_\varepsilon = 0$. Equilibrium of eq. 1 is simply given by $S_0 = \ln \alpha_*$ (hence, population viability requires $\alpha_* > 1$). We now linearize the dynamics (e.g., May et al. 1974; Botsford 1992; Mertz and Myers 1996): small perturbations about equilibrium, $s_t = S_t - S_0$, evolve in accord with the equation

$$(3) \quad s_{t+4} = (1 - \ln \alpha_*) s_t.$$

A particular solution to eq. 3 is immediately obtainable. (In this context, particular means that the solution is not the general solution, which is given below). It is

$$(4) \quad s_{4n} = (1 - \ln \alpha_*)^n s_0$$

where n is an integer and s_0 is the initial population size (note that a separate s_0 must be specified for each of the four lines comprising the salmon population).

From eq. 4, we can immediately conclude that there is a period transition at $\ln \alpha_* = 1$ or, equivalently, $\alpha_* = e$. For $\alpha_* < e$, the first four abundances will be repeated indefinitely (with a superimposed damping); in other words, there will be a 4-year periodicity. For $\alpha_* > e$, the bracketed term in eq. 4 is negative, so that fluctuations change sign every generation (every 4 years), and thus the fundamental period is now 8 years.

This difference equation (eq. 3) can be recast as an algebraic equation (the so-called characteristic equation) using the substitution $s_{t+i} = \chi^i s_t$. Specifically, eq. 3 becomes

$$(5) \quad \chi^4 - (1 - \ln \alpha_*) = 0.$$

The significance of the characteristic equation lies in the fact that all four roots of eq. 5 are necessary to construct the general solution of eq. 3, which will be given in the next paragraph. In Fig. 2 the four roots are plotted in the complex plane for four different α_* values. The transition referred to above is readily apparent in this plot, with a roots-on-axes pattern for $\alpha_* < e$ in contrast with a roots-on-diagonals pattern for $\alpha_* > e$.

The general solution to eq. 3 involves four arbitrary constants, one associated with each root, and these constants are determined by the initial population size of each of the four lines, which must be specified. Roots not lying on the real axis appear as complex conjugate pairs (reflections about the real axis), and these combine to yield real (as opposed to complex)

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frequencies. The principle is illustrated in appendix J of Bulmer (1994). This being so, the general solution to eq. 3, for $\alpha_* < e$, can be written as

$$(6) \quad s_t = |\chi|^t \{a_1 \sin(2\pi t/2) + a_2 \sin[(2\pi t/4) + \theta_2] + a_3\}$$

where a_1, a_2, a_3, and θ_2 are all real constants that are determined by the four initial conditions and $|\chi|$ is the modulus of χ, and from eq. 5, it is clearly given by $|\chi| = (1 - \ln \alpha_*)^{1/4}$. The distance of each root from the origin, in Fig. 2, is equal to $|\chi|$. For $\alpha_* > e$ the general solution is

$$(7) \quad s_t = |\chi|^t \{a_1 \sin[(2\pi t/(8/3)) + \theta_1] + a_2 \sin[(2\pi t/8) + \theta_2]\}$$

where the constants again are determined by the initial conditions.

It is evident from eqs. 6 and 7 that $|\chi|$ acts as an attenuation or amplification factor, depending on whether it is less than or greater than 1, respectively. In the case of the 4-year mode, $|\chi|$ is always less than 1, since $|\chi| = (1 - \ln \alpha_*)^{1/4}$; therefore, α_* must less than e for the 4-year mode to exist. However, as α_* approaches 1, $|\chi|$ approaches 1, and there is no damping (in the linear limit). This allows extremely large responses to stochastic forcing. Since α_* is forced down toward 1 as the harvest rate increases, it follows that high removal rates predispose sockeye populations to energetic cycling.

Instability exists if $|\chi| > 1$ (the roots in Fig. 2 would lie outside the unit circle); the amplitude of the oscillations grows with time. For this to occur, one must have $\alpha_* > e^2 \approx 7.39$, which defines the necessary condition for instability (and thus the 4-year mode can never be unstable, since it exists only for $1 < \alpha_* < e$).

The solution (eq. 6) reveals the presence of a 2-year mode in addition to the 4-year mode. It corresponds to the root on the negative real axis in Fig. 2. (For the roots on or above the real axis, the corresponding period is given by $2\pi/\psi$, where ψ is the angular coordinate of the root with respect to the real axis.) Note that there is no specific phase parameter in eq. 6 for the 2-year mode; the phase of this mode is solely determined by the sign of a_1. The 2-year mode may exist in the actual sockeye data: Fig. 1 reveals that the early Stuart run shows a perceptible oscillation at 2 years period. However, as will become apparent, this 2-year mode could be the result of delayed density dependence.

The solution (eq. 7) reveals a mode with a period of 8/3 years, in addition to the 8-year mode. Like the 8-year mode, this 8/3-year mode changes sign every 4 years.

Prior work (e.g., Botsford 1992; Mertz and Myers 1996) has concentrated on the spontaneous oscillations generated when the equilibrium becomes unstable. As noted above, instability cannot occur for the 4-year mode. In contrast, the 8-year mode will appear spontaneously if α_* is made large enough. This is a Ricker oscillation, i.e., it is our simplified model's version of the oscillations discovered in simulations by Ricker (1954). Emphasis on these spontaneous oscillations has apparently resulted in the overlooking of the transition noted above, and thus the 4-year mode seems to have escaped notice.

For $\sigma_\varepsilon > 0$, oscillations will exist in the absence of instability. The white noise forcing (ε_t) will excite the modes associated with the roots described above. For any given mode, the strength of the oscillations elicited by the noise forcing depends on the proximity of the root (representing the mode) to the unit circle. (When a root lies on the unit circle, the linearized dynamics predict an infinite amplitude for that mode.) For the 4-year mode, the root representing it nears the unit circle as α_* approaches 1; thus, α_* may play a pivotal role in the dynamics of the 4-year cycle. With this in mind, we will now proceed to simulate the response evoked by stochastic forcing.

Simulation results

Meaningful simulation of the sockeye cycles requires plausible estimates of α_* and σ_ε for entry into the model. Ricker (1987) estimated α_* for the aggregated British Columbia sockeye stocks for the period 1950–1972 by, in effect, regressing S_{t+4} on S_t. His analysis indicates that α_* is hardly distinguishable from 1. We applied a similar procedure to each stock and found a mean (over stocks) value of 1.5 for α_*. It is known (Walters 1985; Myers and Barrowman 1995) that there is an upward bias in these estimates. With these facts in mind, we chose $\alpha_* = 1.2$ and $\sigma_\varepsilon = 0.8$ (also derived from the regression analysis) for our baseline case. This analysis is intended only to provide guidance for simulation; we make no pretense to statistical rigor.

In Fig. 3, we present eight simulated series of the baseline case, each with a length of 40 years (comparable with the time spans in Fig. 1). (To remove starting transients the first 100 years of each run were discarded.) The time series and their spectra show a striking resemblance to the true series and their spectra (Fig. 1). For $\alpha_* = 1.2$, almost any 40-year realization of eq. 1 will show a pronounced 4-year oscillation. (For $\alpha_* < 1.2$ the oscillations are more energetic than those shown.) As α_* grows, the 4-year oscillations become more episodic. Additional simulations (some are shown in Figs. 5 and 6, to be discussed later) reveal that the 4-year oscillations decline in strength as α_* increases.

Our conclusion, from these and extensive additional simulations, is that the British Columbia sockeye cycles can be faithfully reproduced by the model with $\alpha_* = 1.1$ to 1.4 (approximately). This suggests an important role for harvest rate: the natural reproductive parameter, α, is too large (Myers and Barrowman 1995), about 5, to permit cycling with a 4-year period, but with a typical H of 0.75 to 0.8 (Cass and Wood 1994), the realized reproductive parameter $\alpha_* = (1 - H)\alpha$ has the ideal magnitude for strong cycling. Indeed, any increase in H is likely to lead to more violent oscillations, an unwelcome outcome.

Our finding that chance events can create cycles differs from that reached by Cass and Wood (1994). They concluded that simulations could not reproduce the range of abundances observed; however, their comparison might be biased because they used a subset of the most cyclic stocks for their comparison and averaged their simulation output. Our results clearly show that there is wide variability among runs, making comparisons among averages difficult.

It should be emphasized that a constant H (of about 0.75) is sufficient to account for the cycling, and thus, depensatory fishing (e.g., Levy and Wood 1992) need not be invoked (depensatory harvesting implies an H that has an inverse dependence on population size). The aboriginal fishery may operate in a depensatory manner (Peterman 1980; Ricker 1987), which, prior to the development of an intense commercial fishery, may have enhanced the natural tendency to cycle at a

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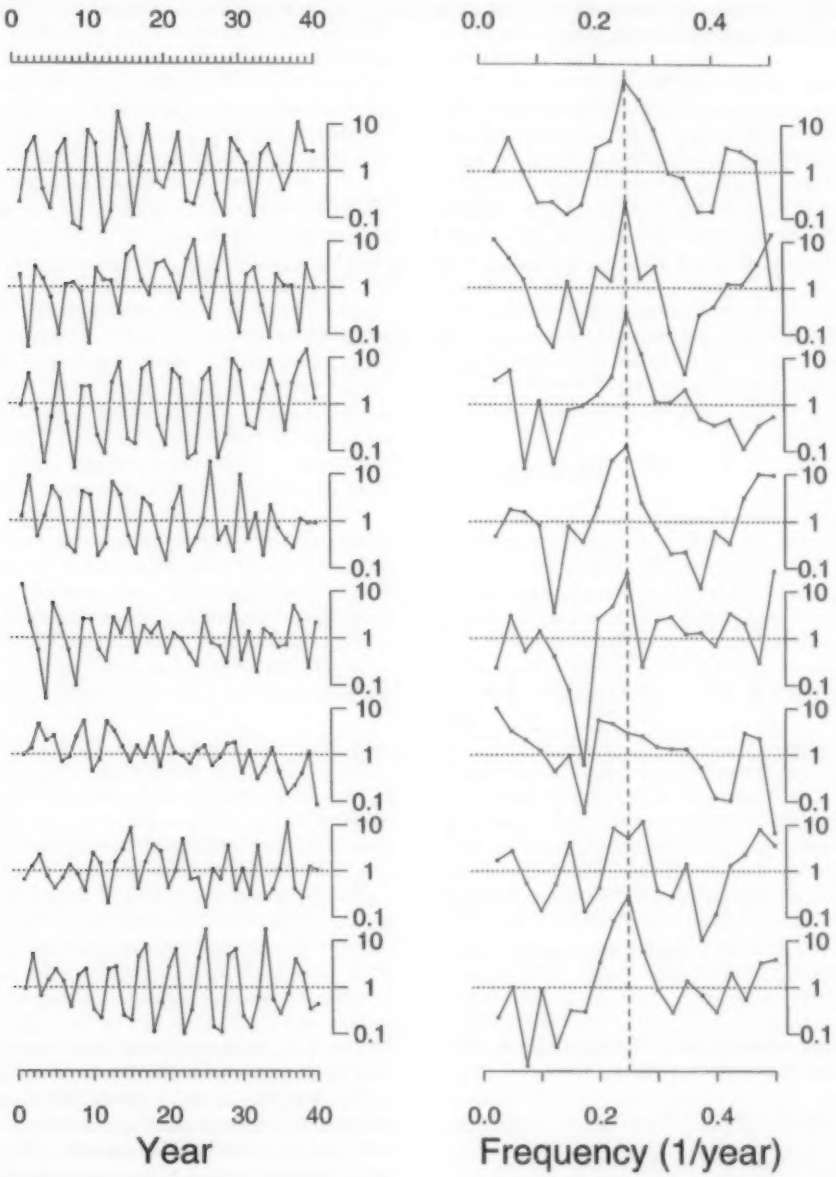
Fig. 3. Eight realizations of eq. 1, given as log-transformed spawner abundance, and their spectra for $\alpha_s = 1.2$ and $\sigma = 0.8$. See Fig. 1 for details.



4-year period (Levy and Wood 1992). However, for the present era, recent analysis (Cass and Wood 1994) suggests that fishing is not depensatory. Similarly, there appears to be no evidence that depensatory natural mortality is occurring at the present (Myers et al. 1995).

An important finding is that the sockeye cycles can occur in the complete absence of delayed density dependence. This does not, of course, establish the absence of delayed density dependence in the British Columbia sockeye populations, and in the next section, we will examine the effect of delayed density dependence on the cycles produced by our model.

Delayed density dependence

We investigated the influence of delayed density dependence, which was included in the model by rewriting eq. 1 following Larkin (1971) as

$$(8) \quad S'_{t+4} = \alpha_s S'_t \exp(-\beta S'_t - \phi' S'_{t-1} - \gamma' S'_{t-2} + \varepsilon_t)$$

where ϕ' and γ' (both must be positive) are the coefficients for density dependence acting at a lag of 1 and 2 years, respectively (e.g., see Collie and Walters 1987). We again scale with

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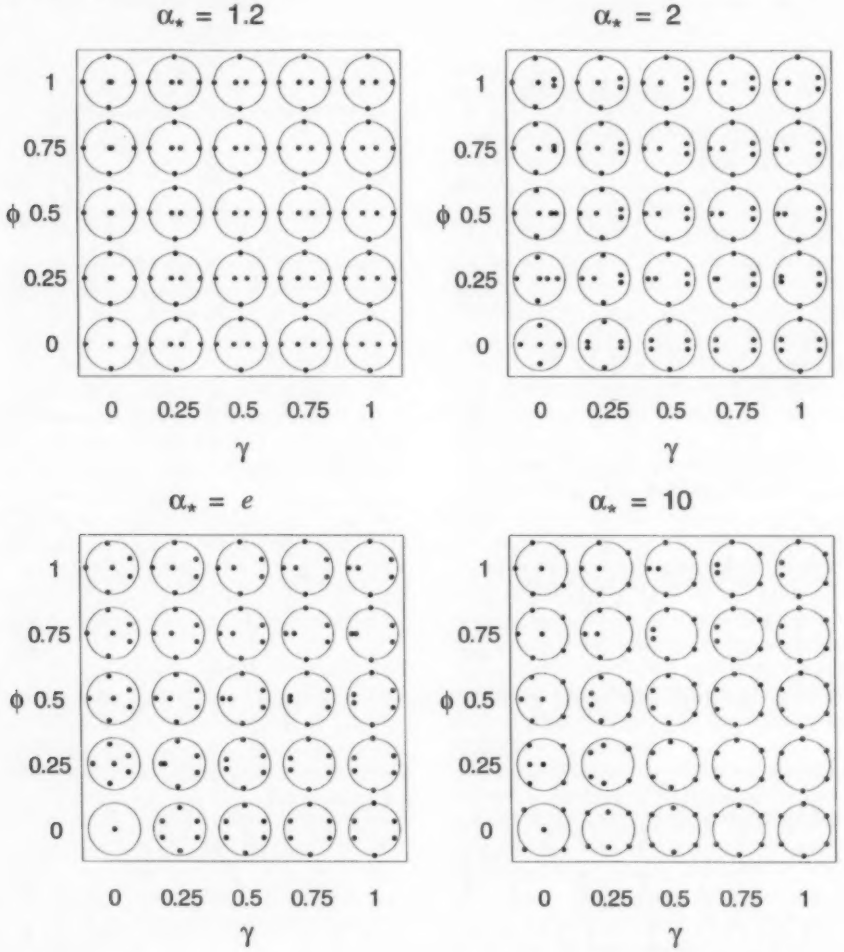
Fig. 4. Complex roots of a model with two lagged density-dependent mortality terms (eq. 9) and an age of maturity of 4 as a function of density-dependent mortality caused by the number of spawners in the previous cohort in year S_{t-1} (ϕ), the number of spawners 2 years previously, S_{t-2} (γ), and α. For each set of parameters (ϕ, γ, and α) the roots are shown in relationship to the unit circle. The real and imaginary axes, as shown in Fig. 2, are implicit in these diagrams.



β, forming the normalized coefficients of delayed density dependence $\phi = \beta\phi'$ and $\gamma = \beta\gamma'$, and rewrite eq. 5 as

$$(9) \quad S_{t+4} = \alpha_* S_t \exp(-S_t - \phi S_{t-1} - \gamma S_{t-2} + \varepsilon_t)$$

This equation forms the basis for the analysis to follow.

The analysis of the modal structure closely parallels that given for the case of no delayed density dependence. The equilibrium is now given by $S_0 = \ln \alpha_*/(1 + \phi + \gamma)$. Small perturbations to equilibrium, $s_t = S_t - S_0$, evolve according to

$$(10) \quad s_{t+4} = \left(1 - \frac{\ln \alpha_*}{1+\phi+\gamma}\right) s_t - \frac{\phi \ln \alpha_*}{1+\phi+\gamma} s_{t-1} - \frac{\gamma \ln \alpha_*}{1+\phi+\gamma} s_{t-2}$$

and the characteristic equation is

$$(11) \quad \chi^6 - \left(1 - \frac{\ln \alpha_*}{1+\phi+\gamma}\right)\chi^2 + \frac{\phi \ln \alpha_*}{1+\phi+\gamma}\chi + \frac{\gamma \ln \alpha_*}{1+\phi+\gamma} = 0.$$

The roots of the characteristic equation can again be used to find the fundamental modes for the population dynamics.

The dependence of the modal structure on ϕ and γ is depicted in Fig. 4. Each panel is characterized by a single value of α_*, and each circle within a panel corresponds to a ϕ, γ pair. Each circle is identical to the circles shown in Fig. 2, that is, each is a unit circle and the roots of eq. 8 are shown in relation to it. Note that there are, in general, six roots for each case, although there are examples where two or more roots have coalesced so that fewer than six roots appear.

The range shown for ϕ and γ is significant. Of course, $\phi = 0$ implies no lag-one density dependence and $\gamma = 0$ implies no lag-two density dependence. At the other extreme, $\phi = 1$ would indicate that lag-one density dependence is as strong as direct (unlagged) density dependence, and similarly for $\gamma = 1$.

As in Fig. 2, the proximity of a root to the unit circle is particularly important. For example, in the lower right panel, for the case $\phi = \gamma = 0$, there are clearly roots outside the unit

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circle, indicating the presence of instability (the classic 8-year Ricker oscillations arise spontaneously in this case).

Consider first the top two panels of Fig. 4, for which $\alpha_s < e$. Similarly to Fig. 2, when $\alpha_s < e$, the roots tend to be aligned with the real and imaginary axes. The effect of lag-one density dependence (ϕ) is to push the 2-year mode (the root on the negative real axis) closer to or beyond the unit circle, increasing the propensity to cycle at this period. The lag-two density dependence (γ) has a similar effect on the 4-year mode (the root on the positive imaginary axis).

For the panel labeled $\alpha_s = e$ (this is the transitional case) the delayed density dependence destroys the degeneracy of the $\phi = \gamma = 0$ case (all roots coalesced at origin). As ϕ and γ increase, the 2- and 4-year modes, respectively, are again enhanced, in the sense that their roots approach the unit circle.

The last panel, labeled $\alpha_s = 10$, illustrates a case for which a Ricker oscillation (represented by the root in the first quadrant) is expected for $\phi = \gamma = 0$. The effect of lag-one density dependence is particularly dramatic, in that it completely alters the pattern of roots, replacing the unstable Ricker mode with an unstable 2-year mode, as ϕ approaches 1. The lag-two density dependence tends to stabilize the Ricker mode and, most importantly, gives rise to a 4-year mode.

To summarize Fig. 4, the root plots suggest that the general tendency of the lag-one density dependence is to predispose the population to oscillations with a 2-year period, and, similarly, the lag-two density dependence enhances 4-year period oscillations; this is the intuitive result first suggested by Ricker (1950). These conclusions are borne out by simulations, a number of which are presented in Figs. 5 and 6, and are discussed below.

To guide the simulations of the model with delayed density dependence, we referred to the work of Myers et al. (1997), who used metaanalysis to estimate the strength of lag-one and lag-two density dependence for all available (34 time-series) sockeye fry, smolt, and spawner data. They found that there was no detectable lag-two density dependence, but at lag one, there was detectable density dependence with a strength of $\phi \approx 0.2$ (i.e., the lag-one effect was about 20% of the direct density dependence). In the simulations discussed below, when we apply delayed density dependence, we will set $\phi = 0.3$ and (or) $\gamma = 0.3$ so that its strength is at the upper limit of plausibility.

In Fig. 5, we display a set of 100-year simulations portraying the influence of delayed density dependence; σ_ε is fixed at the standard value of 0.8 in all cases. The most consistent aspect of these plots is the tendency of the strength and regularity of the 4-year oscillations to decline as α_s increases. The influence of delayed density dependence is not obvious here: we would expect the most vigorous 4-year oscillations to occur under the effect of lag-two density dependence; instead, the strongest oscillations appear for one of the lag-one cases (1.2, 0.3, 0.0). This appears to be a manifestation of realization to realization variability, which can be enormous.

In Fig. 6, we see that a modest enhancement in the level of stochastic forcing, from $\sigma_\varepsilon = 0.8$ to $\sigma_\varepsilon = 1.0$, produces far more vigorous oscillations than those appearing in Fig. 5. (A value for σ_ε of 1.0 is by no means excluded by the data analysis.) The topmost series is of great interest, in that the oscillations span four orders of magnitude, as great as the range of any observed sockeye series. And, it should be noted, this is in the absence of delayed density dependence.

With the stronger forcing imposed in the Fig. 6 simulations, the effect of delayed density dependence is now more apparent. For example, even with a large α_s of 3.0, energetic 4-year oscillations are produced under the influence of lag-two density dependence.

To summarize this section, realistic levels of delayed density dependence do not appear to greatly modify the cycles that appear under stochastic forcing in its absence. At a forcing level of $\sigma_\varepsilon = 0.8$, realization to realization variability seemed to obscure the influence of delayed density dependence. At a slightly higher forcing level of $\sigma_\varepsilon = 1.0$ the effect of delayed density dependence was more apparent. However, with forcing of this strength, the oscillations, under the influence of delayed density dependence, are more intense and more persistent than those observed. It is conceivable that lag-two density dependence plays a reinforcing role in the observed cycles, but this effect is not necessary to generate them. (We must again note that Myers et al. (1997) found that there was no evidence of a significant lag-two effect in their metaanalysis.)

Conclusions

From a population dynamics perspective, there are two interesting aspects of our analysis. (*i*) The 4-year oscillation can be regarded as a stable companion mode of the well-known unstable Ricker (8-year) mode. (*ii*) The realized reproductive parameter, α_s, controls both the period of the oscillation and its amplitude (jointly with σ_ε). The 4-year oscillations appear to be inevitable at sufficiently high harvest rates, and they do not require delayed density dependence or depensatory fishing.

We propose that the population trajectories of the major Fraser River sockeye populations (Fig. 1) could be thought of as different realizations from the $\alpha_s \approx 1$ dynamical regime, requiring no particular mechanism for any individual case. Cycles are established by chance events, and the dynamics of the system tend to promote their persistence. Thus the diversity of patterns among the Fraser populations is expected, as are the occasional shifts in the cyclic patterns caused by environmental events. Examples of such shifts are provided by the exceptionally strong returns of the Adams Lake subdominant run in the 1960s (Levy and Wood 1992). These runs were deliberately fished down to restore the previous pattern; without this intervention, it seems likely that the resulting cycle pattern may have been quite different. Recently, the fertilization of Chilko Lake appears to have caused an increase in abundance of the once formerly low-abundance lines to be comparable in abundance with the others, eliminating the cyclic pattern in this population (Ricker 1997). Similarly, the dominant line of the Scotch Creek (Shuswap Lake) population has recently shifted by 1 year.

There is increasing evidence that the effects of large juvenile populations on lake zooplankton populations can carry over and affect the survival or growth of subsequent populations (Myers et al. 1997; Koenings and Kyle 1998). Our analysis suggests that such delayed density dependence may make a small contribution to population cycles; however, the role of delayed density dependence may increase if populations continue to grow to meet or exceed lake carrying capacity (e.g., Hume et al. 1996).

Are our results sufficient to explain all the aspects of the

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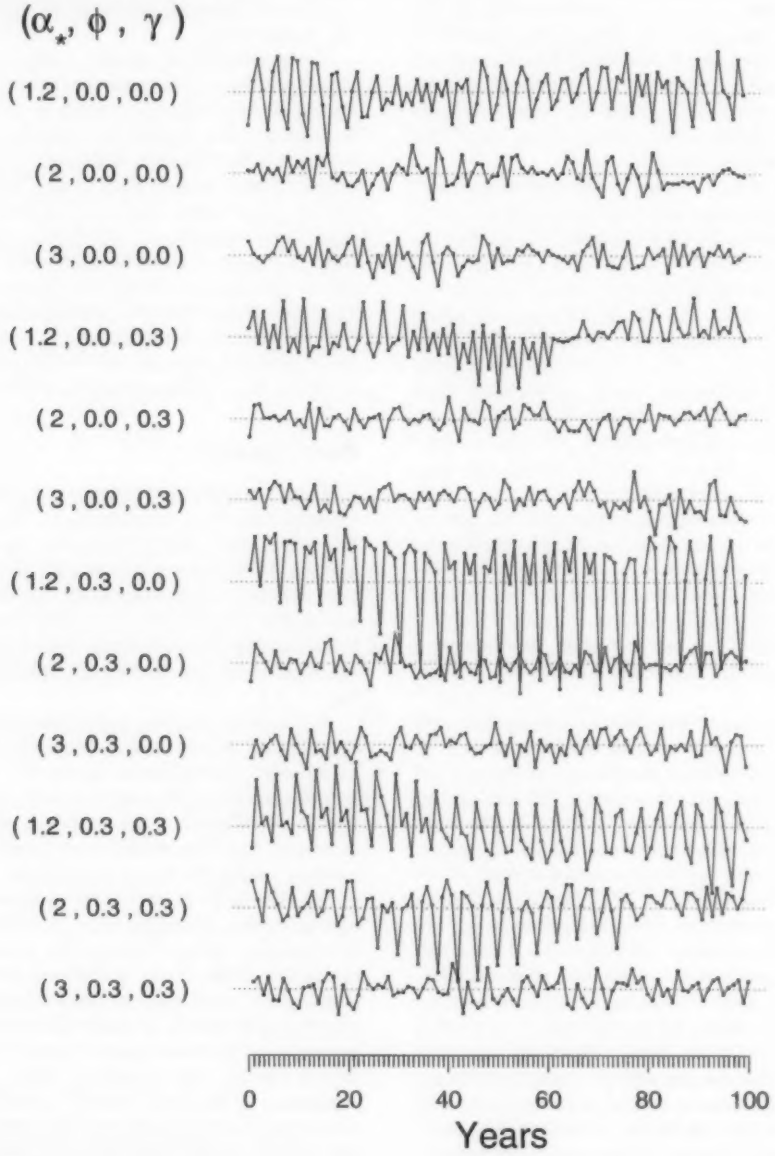
Fig. 5. Simulations of log-transformed spawner abundance, based on eq. 9, each of 100 years duration. The horizontal lines are offset by 3 orders of magnitude (note that this is a larger scale than used in Fig. 1). The bracketed triplets on the left give the α_*, ϕ, γ values, as indicated. For all of these runs the noise level was set at $\sigma_\varepsilon = 0.8$.



observed cycles in the Fraser River? This is a difficult question because we only have a limited number of realizations and the dynamics of the low-abundance lines are inherently difficult to observe. It is certainly possible that subtle changes in fishing on the nondominant lines could keep the low lines down, and it would be difficult to estimate (Cass and Wood 1994). Furthermore, the occasional management decision to increase harvest on the subdominant lines (Cass and Wood 1994) certainly enhanced the observed cycles.

The difficulty of separating the role of chance from deterministic phenomena always creates difficulties. This is perhaps at the heart of how our view of the Fraser sockeye differs from Ricker's (1997). Our view is that the present cycles in the Fraser River should be best viewed as realizations of a stochastically forced dynamical system that inevitably produces 4-year cycles at high harvest rates. A combination of chance, biology (e.g., delayed density dependent mortality), and past management decisions to increase harvest on subdominant lines has resulted in the presently observed cycles. Ricker (1997) puts more emphasis on the importance of deterministic biological interactions than we do. This difference in interpretation is perhaps caused by the fact that the importance

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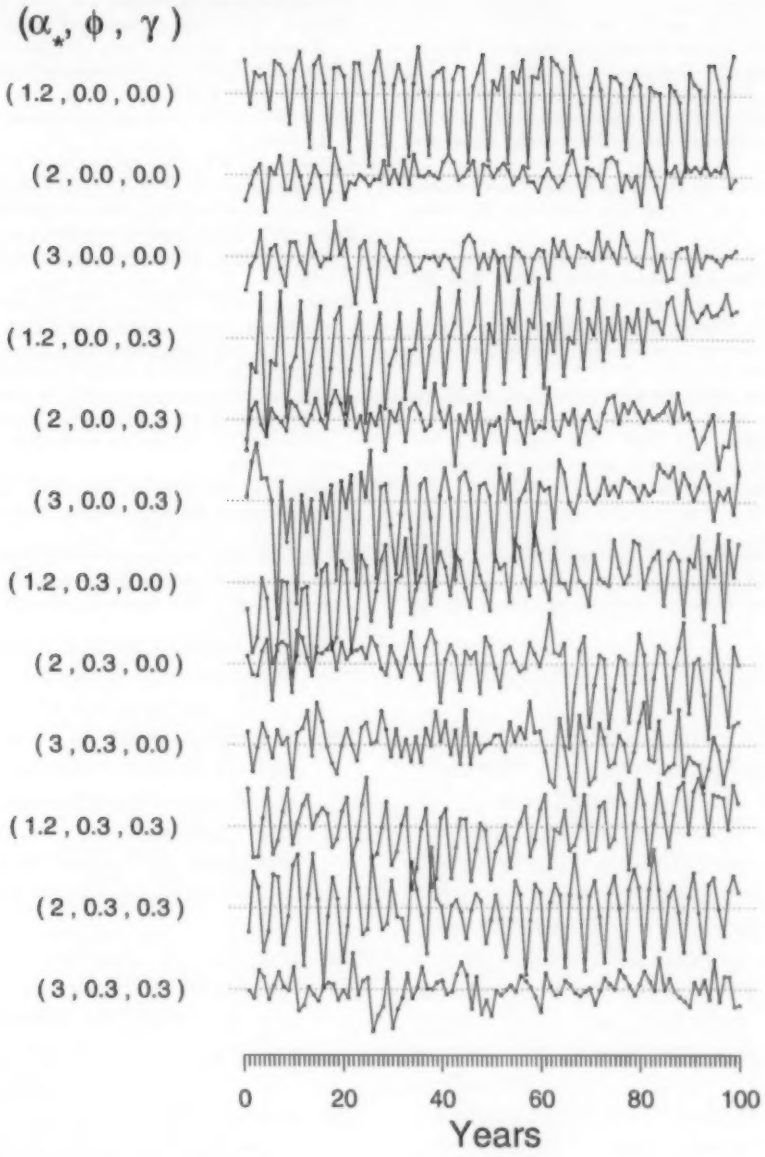
Fig. 6. As in Fig. 5, but with the noise level now fixed at $\sigma_e = 1.0$.



of the stable 4-year dynamical mode has been previously unrecognized.

Finally, from a management perspective, our results are consistent with previous studies (e.g., Welch and Noakes 1990) that suggested that a slight relaxation in harvest rates on nondominant years should result in an increase in yield and a corresponding decrease in the cyclic tendency in these populations.

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References

Botsford, L.W. 1992. Further analysis of Clark's delayed recruitment model. Bull. Math. Biol. **54**: 275–293.

Bulmer, M. 1994. Theoretical evolutionary ecology. Sinauer, Sunderland, Mass.

Cass, A.J., and Wood, C.C. 1994. Evaluation of the depensatory fishing hypothesis as an explanation for population cycles in Fraser River sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. **51**: 1839–1854.

Collie, J.S., and Walters, C.J. 1987. Alternative recruitment models

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of Adams River sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Sci. **44**: 1551–1561.

Hume, J.M.B., Shortreed, K.S., and Morton, K.F. 1996. Juvenile sockeye rearing capacity of three lakes in the Fraser River system. Can. J. Fish. Aquat. Sci. **53**: 719–733.

Koenings, J.P., and Kyle, G.B. 1998. Collapsed populations and delayed recovery of zooplankton due to intense foraging by juvenile sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. **55**. In press.

Larkin, P.A. 1971. Simulation studies of the Adams River sockeye salmon, *Oncorhynchus nerka*. J. Fish. Res. Board Can. **28**: 1493–1502.

Levy, D.A., and Wood, C.C. 1992. Review of proposed mechanisms for sockeye salmon cycles in the Fraser River. J. Math. Biol. **54**: 241–261.

May, R.M., Conway, G.R., Hassell, M.P., and Southwood, T.R.E. 1974. Time delays, density-dependence, and single species oscillations. J. Anim. Ecol. **43**: 747–770.

Mertz, G., and Myers, R.A. 1996. An augmented Clark model for stability of populations. Math. Biosci. **131**: 157–171.

Myers, R.A., and Barrowman, N.J. 1995. Time series bias in the estimation of density-dependent mortality in stock–recruitment models. Can. J. Fish. Aquat. Sci. **52**: 223–232.

Myers, R.A., Barrowman, N.J., Hutchings, J.A., and Rosenberg, A.A. 1995. Population dynamics of exploited fish stocks at low population levels. Science (Washington, D.C.), **269**: 1106–1108.

Myers, R.A., Bradford, M.J., Bridson, J.M., and Mertz, G. 1997. Estimating delayed density-dependent mortality in sockeye salmon (*Oncorhynchus nerka*): a meta-analytic approach. Can. J. Fish. Aquat. Sci. **54**: 2449–2462.

Peterman, R.M. 1980. Dynamics of native food fisheries on salmon in British Columbia. Can. J. Fish. Aquat. Sci. **37**: 561–566.

Ricker, W.E. 1950. Cyclic dominance among the Fraser sockeye. Ecology, **31**: 6–26.

Ricker, W.E. 1954. Stock and recruitment. J. Fish. Res. Board Can. **11**: 559–623.

Ricker, W.E. 1987. Effects of the fishery and of obstacles to migration on the abundance of Fraser River sockeye salmon (*Oncorhynchus nerka*). Can. Tech. Rep. Fish. Aquat. Sci. No. 1792.

Ricker, W.E. 1997. Cycles of abundance among Fraser River sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. **54**: 950–968.

Walters, C.J. 1985. Bias in the estimation of the functional relationships from time series data. Can. J. Fish. Aquat. Sci. **42**: 147–149.

Walters, C.J., and Staley, M.J. 1987. Evidence against the existence of cyclic dominance in Fraser River sockeye salmon (*Oncorhynchus nerka*). *In* Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. *Edited by* H.D. Smith, L. Margolis, and C.C. Wood. Can. Spec. Publ. Fish. Aquat. Sci. No. 96. pp. 375–384.

Walters, C.J., and Woodey, J.C. 1992. Genetic models for cyclic dominance in sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. **49**: 281–292.

Welch, D.W., and Noakes, D.J. 1990. Cyclic dominance and optimal escapement of Adams River sockeye salmon (*Oncorhynchus nerka*). Can. J. Fish. Aquat. Sci. **47**: 838–849.

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